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A Summary of Current Program and Preliminary Report of Progress

A280, 2409 Ag8 1961/62

LIVESTOCK RESEARCH

of the
United States Department of Agriculture
and cooperating agencies

This progress report of U.S.D.A. and cooperative research is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

There is included under each problem area in the report, a brief and very general statement on the Nature of the research being conducted by the State Agricultural Experiment Stations and the professional manpower being devoted by the State stations to such research. Also included is a brief description of related work conducted by private organizations. No details on progress of State station or industry research are included except as such work is cooperative with U.S.D.A.

The summaries of progress on U.S.D.A. and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having an interest in the development of public agricultural research programs.

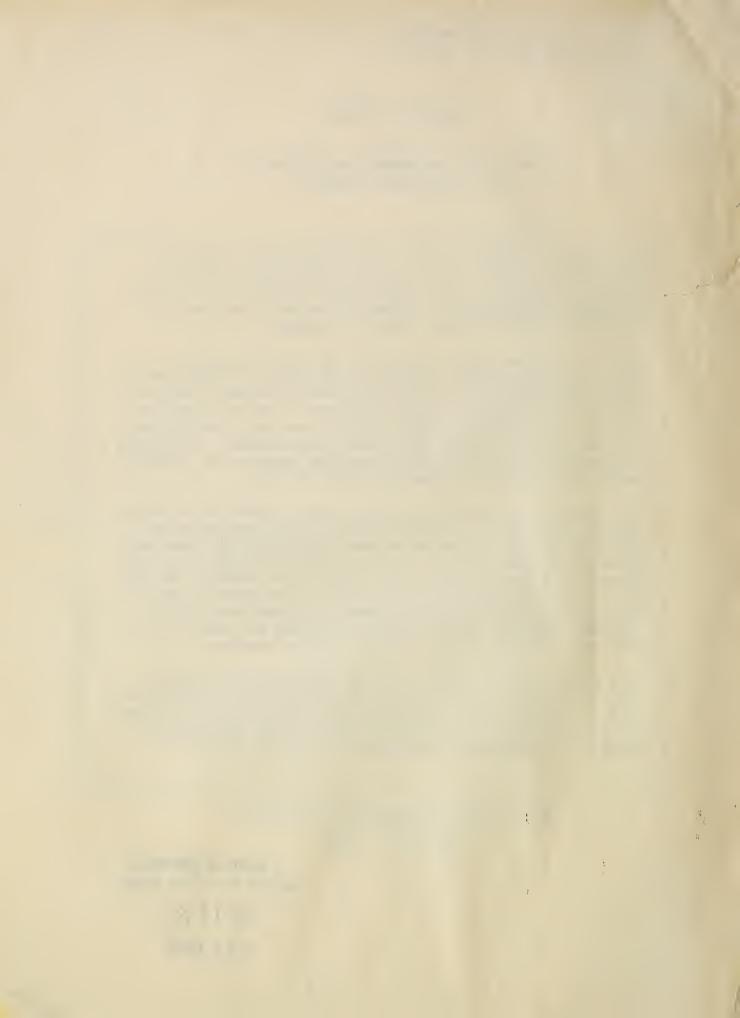
This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued during the last two years. Current agricultural research findings are also published in the monthly U.S.D.A. publications, Agricultural Research, Agricultural Marketing, and The Farm Index.

UNITED STATES DEPARTMENT OF AGRICULTURE Washington, D. C.
January 15, 1963

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#### OTHER COMMODITY AND FUNCTIONAL REPORTS

A progress report similar to this one is prepared for use by each of the following research and marketing advisory committees:

Citrus and Subtropical Fruit
Cotton and Cottonseed
Dairy
Deciduous Fruit and Tree Nut
Forage, Feed and Seed
Forestry
Grain
Oilseeds and Peanut

Potato Poultry Rice Sheep and Wool

Sugar Tobacco Vegetable Economics

Farm Equipment and Structures

Food and Nutrition Food Distribution Home Economics

Soils, Water and Fertilizer Transportation and Storage

Two additional reports of progress are prepared in order to make available the complete research program. They are:

Ornamentals and Other Miscellaneous Commodities Other Research — Cross Commodity

#### ORGANIZATIONAL UNIT REPORTS

All of the material in the commodity and functional reports listed above is the same as that found in the 20 division and 3 service research reports listed below.

### Agricultural Research Service (ARS)

Agricultural Engineering Animal Disease and Parasite Animal Husbandry

Crops

Entomology

Soil and Water Conservation

Utilization -- Eastern
Utilization -- Northern
Utilization -- Southern
Utilization -- Western

Human Nutrition

Clothing and Housing

Consumer and Food Economics

## Agricultural Marketing Service (AMS)

Market Quality
Transportation & Facilities

## Economic Research Service (ERS)

Farm Economics
Marketing Economics
Economic & Statistical Analysis
Foreign Development and Trade Analysis
Foreign Regional Analysis

## Other Services

Farmer Cooperative Service (FCS)
Forest Service (FS)
Statistical Reporting Service (SRS)

A copy of this report or any of the others listed above may be requested from Max Hinds, Executive Secretary, Livestock Research and Marketing Advisory Committee, Agricultural Research Service, U. S. Department of Agriculture, Washington 25, D. C.

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#### INTRODUCTION

Livestock research as used in this report is concerned with the production, processing, marketing, and consumption of livestock and livestock products. The report covers Farm Research, Utilization Research, Marketing Research, Economic Research, and Consumer-Use Research of the USDA and cooperating agencies. Only a brief description of the related work of the State Experiment Stations and industry is included.

Under each of the Problem Areas there is a statement describing the Program of work underway and the professional man-years devoted to the major kinds of research included. The relative scope of the total research effort pertaining to livestock and livestock products is indicated by the approximate number of professional man-years employed: 260 by USDA, 417 by the State Experiment Stations and about 1,380 by industry and other organizations.

A brief report of Progress and significant findings for USDA and cooperative programs is given for each phase of the research program. A considerable amount of basic cross-commodity and functional research that will supply new knowledge applicable to livestock and meat problems is not included in this report. Such research is included in the functional reports such as "Economics," "Soils, Water and Fertilizer," and in the Other Research" report.

## Research by USDA

The farm research pertaining to cattle and hogs comprises investigations of breeding, physiology, nutrition, diseases and parasites, housing, equipment, management and production influences on animal products. This research is conducted by the following divisions of Agricultural Research Service: Animal Husbandry, Animal Disease and Parasite, Agricultural Engineering and Entomology. In fiscal year 1962 this work involved about 120 professional man-years compared with about 300 at State experiment stations and 500 for industry.

Utilization research deals with the processing phase involving slaughter, cutting, trimming, smoking, curing, and preparing for later use by consumers. The work includes considerable emphasis on chemical and physical properties of meat. Also, it is concerned with improved equipment and processes. The work is done at the Eastern Utilization Research and Development Division, Wyndmoor, Pennsylvania, and at Beltsville, Maryland, and under contract with State and foreign-country laboratories and in cooperation with the industry and other organizations mentioned under Program for each research area. In fiscal year 1962 the work involved about 100 professional man-years, compared with about 30 at State Experiment Stations and 400 estimated in industry.

Marketing research involves the physical and biological aspects of assembly, packaging, transporting, storing, and distribution from the time the product leaves the farm until it reaches the ultimate consumer. The work reported herein is conducted by two divisions in the Agricultural Marketing Service: Transportation and Facilities and Market Quality. In fiscal year 1962 this work involved about 8 professional man-years compared with 8 at State experiment stations and an estimated 175 for industry.

Economic research is concerned with marketing costs, margins and efficiency, market potential, supply and demand, outlook and situation, and improving marketing through research with farmer cooperatives. Fundamental research contributes tools for determining elasticities of demand, statistical formulas, and other analytical guides that can be applied to different situations. The work reported herein is done by the Economic and Statistical Analysis and the Marketing Economics Divisions of the Economic Research Service, and by the Marketing Division of the Farmer Cooperative Service. Approximately 25 professional man-years were devoted to this work in fiscal year 1962 compared with about 60 at State experiment stations. Work by industry in this field is usually on a functional basis and a reliable estimate for livestock and meat is not available.

Nutrition and Consumer-use research pertains to composition and nutritive value; physiological availability of nutrients and their effects; and new and improved methods of preparation, preservation, and care in homes, eating establishments and institutions. This work is done by the divisions of Human Nutrition Research and Consumer and Food Economics Research of the Agricultural Research Service. In fiscal year 1962 this work involved approximately 11 professional man-years compared with 10 at State experiment stations. A reliable estimate of work by industry pertaining specifically to beef and pork is not available.

## Research by State Experiment Stations

There is included under each problem area a brief and very general statement on the nature of the research being conducted by the State Agricultural Experiment Stations and the professional manpower being devoted by the State stations to such research.

Consolidating this information for the entire field of interest we find that in fiscal year 1962 a total of 417.2 professional man-years were spent by the State Agricultural Experiment Stations on livestock research.

Livestock research in 1962 was in progress in 47 of the 53 State Agricultural Experiment Stations. Studies underway were carried out by research workers in departments of Animal Science, Agricultural Econo-

mics, Agricultural Engineering, Entomology, Food Technology, Home Economics, and Veterinary Science.

Livestock research at the State Agricultural Experiment Stations included 9.1 man-years on equipment and buildings used in beef and swine production; 269.4 man-years on beef cattle and swine breeding, physiology, nutrition and management; 21.4 man-years on diseases and parasites (not including 94.9 man-years of cattle disease and parasite research reported to the Dairy Research and Marketing Advisory Committee); ll.1 man-years on livestock insects; 27.1 man-years on utilization research for meats, animal fats and oils; 9.5 man-years on human nutrition and consumer-use research; 8.1 man-years on market quality of livestock and meat; and 61.5 man-years of livestock marketing and economics research. In addition, 92.8 man-years of research were conducted in the area of animal biology and cannot be readily identified as to commodity. Other related studies which cannot be identified specifically as livestock research but which have application in the field includes work on leather products and processing, transportation, marketing facilities and protection against radioactive fallout.

No details on progress of State station research are included in this report except as such work is cooperative with USDA

#### Research by Industry and Other Organizations

The 1,380 professional man-years estimated as industry's participation in livestock research are employed primarily by feed and equipment manufacturers, meat processors, and chemical companies. Feed manufacturers have placed major emphasis on studying rations for livestock and the development and testing of new formulations of mixed feeds including chemical growth stimulators.

About 1/3 of industry's contribution to the research effort pertains to farm research. In contrast with the poultry industry where practically all breeding research is done by industry, very little is done by industry in beef, except the work of large firms like the King Ranch which developed the Santa Gertrudis breed. The scope of operation required for successful breeding of beef cattle, because of the size of animal and length of life cycle which tie up a substantial amount of funds, will probably keep such research with public institutions. In the case of swine there is a real opportunity for increased participation by industry. The task of evaluating breeds, the performance of breeds in crossing, and the comparison of crossing systems will take more animals than are available at publicly supported experiment stations. Basic information developed through public research is used by a large number of companies to develop products intended for prevention, control, or treatment of diseases, parasites, and insects. Industry research also tests chemicals and measures residues, however, final evaluation is often done cooperatively with public agencies.

Also, most of the identification and classification of insects, diseases, and parasites is done by public institutions.

About equal to the farm research effort in the livestock industry, another one-third is in the utilization field. In contrast with the public research in basic work the industry program places strong emphasis on developmental activities and solving of immediate problems. The work of meat packers is devoted to finding industrial utilization of by-products, quality control devices, improved formulation of products, improved handling and plant arrangement. Independent laboratories and foundations take on short time problem solving for clients in the meat industry. Pharmaceutical firms carry on research on extraction of biologically active substances from meat by-products such as hormones from glands, and with the development of agents, such as antibiotics for use in meat processing.

Industry is showing an increasing willingness toward cooperative research with public agencies. This is well illustrated in the chemical field where increasing costs, difficulty, and the time required to secure clearance because of residue problems before a new pesticide can be used on food products make it less attractive for the private companies to work alone. Basic research by private companies is now even less attractive than before and makes increasingly evident the necessity for basic research by public agencies. The advantage of cooperative applied research between public and private agencies is well illustrated in the work with stored products insects. Here literally millions of dollars worth of produce, materials, equipment, storage space and additional manpower are made available by industry at no cost to the Department.

It has been estimated that one billion dollars per year is being expended in building new and modernizing old marketing facilities. Planning of marketing facilities that benefit handlers of commodities such as livestock and livestock products flowing through the marketing channels is of such magnitude and affects so many individuals and community interests that its nature makes it a public activity. A small Federal staff makes an important contribution to overall market planning which is utilized by architectural and engineering firms to develop plans, drawings and specifications for specific facilities on particular sites. In contrast with research on facilities, industry research in the equipment field is at ratio of 240 industry man-years to 5 for public research. Engineering research and development is devoted to improving operations of feedlots, auction markets and terminal stockyards, packing houses, and distribution activities.

The greater part of the marketing economic research in industry is conducted in connection with new product development and in merchandizing and promoting farm products. In addition, industry contributes in

a large way to State and Federal research programs by offering its facilities and facts to the public research agencies. Public research is used for comparison and analysis where private research does not have access to the plants and records of competitors. Industry participates heavily in consumer preference research but largely with respect to a firm's own brands.

# Examples of Recent Research Accomplishments by USDA and Cooperating Scientists

Performance testing in beef cattle. In 1961, more than 308,000 cows in over 4,200 herds throughout the United States were enrolled in brood cow performance testing programs. More than 15,000 bulls were tested for gaining ability under feedlot conditions and at least 191 breeders are known to be following systematic progeny testing programs for carcass evaluation. Virtually all this industry application has occurred since 1954 and is a direct outgrowth by research initiated by the Department of Agriculture in 1930 and carried out through regional projects in cooperation with 35 State experiment stations since the late 1940s. Calculations based on heritability estimates and other genetic and phenotypic parameters developed as a result of this research work indicate that if universal application were made of performance testing techniques now available, the following improvements over a 10-year period would be possible (1) increases in weaning weight of from 5 to 10%, (2) increases of more than  $1/\frac{1}{4}$  lb. in average daily gain postweaning, which would be associated with a 6 to 8% saving in feed, and (3) moderate improvements in carcass lean content and tenderness and palatability of resulting beef.

Meat-type hogs. Improved swine carcasses have been the goal of research by the USDA and cooperating State experiment stations through work at Beltsville, Maryland, and the Regional Swine Breeding Laboratory. As a result of these efforts, a growing number of markets are grading hogs on basis of USDA standards based on scientific data indicating the traits affecting pork quality. Breeding research has shown that the yield of lean cuts in meat-type hogs can be greater than 50% as compared to high in fat hogs. Very recent studies have shown that selection for low fat carcasses can decrease back fat thickness by 18% in 7 generations. The additional worth of meat-type animals to the farmer is estimated at \$5.00 per head. The percent of meat-type hogs marketed in the U.S. has risen from 17 in 1956 to 30 in 1961.

Sterility method of insect control. A new concept in controlling insects and other pests has been developed which may contribute to the solution of some major insect problems. The method involves the release of sterile insects for their own destruction. The mass production and release of screw-worm flies made sterile by gamma radiation resulted in the elimination of this important livestock pest from the Southeast. A similar and more complex program based on the same principle is now underway in the Southwest. In addition, the utilization of sterile insects is being intensively investigated as an aid to the control or eradication of other major pests, including tropical fruit flies, boll weevil, pink bollworm, sugarcane borer, coddling moth, tobacco hornworm, and other pests. An important advance in research on the sterility method has been the development of several chemicals which produce sterility in insects with less damage to the insects than that caused by radiation. The availability of such chemicals also offers the possibility of discovering ways to induce sterility in the natural insect population, thus obviating the necessity of rearing and releasing insects sterilized by radiation to achieve control.

Ventilation of livestock buildings. Research in cooperation with

State experiment stations has obtained much needed basic data on the heat of building environment on production and feed consumption. The heat and moisture dissipation data are considered basic design data for ventilation systems of poultry, dairy, and swine buildings. They appear in design handbooks including the 1962 Guide and Data Book of the American Society of Heating, Refrigeration, Ventilating, and Air Conditioning Engineers, and are used by makers of ventilating equipment, prefabricated buildings, and package buildings as well as by specialists advising farmers on their own construction. Building improvements resulting from the above research have contributed to the substantial rise in efficiency of livestock production that has occurred during the past decade.

Industrial chemicals from fats and oils. Basic and applied research in the chemistry of animal fats has led to the industrial use of many chemicals based on animal fats and vegetable oils. These uses include epoxidized oils as stabilizer-plasticizers in vinyl resins, vinyl stearate polymers and copolymers in waxes and water-emulsion paints, derivatives of o-sulfoacids in wetting agents and soap-detergent combinations, improved oleic acid as a raw material for the chemical industry and improved emulsifiers used in the manufacture of synthetic rubber.

The volume of fats and oils going into these markets is estimated at about 100 million pounds per year.

Reducing Livestock Loss and Damage. FCS is studying loss and damage to livestock through handling and transportation during marketing. Farmers and their livestock marketing agencies incur a loss of more than \$50 million each year due to injury, death and other forms of damage associated with the movement of live animals from farms and feedlots to final destinations. One study showed that losses on transporting and handling sheep and lambs amount to \$2 million a year; abusive handling was a principal factor.

## Programs of Marketing Service and Education

The Research and Marketing Act of 1946 authorized a number of activities in addition to research. Some of these are: "to conduct and cooperate in consumer education...to collect, tabulate, and disseminate statistics on marketing agricultural products...to develop and promulgate procurement standards and specifications for agricultural products...to inspect, certify, and identify the class, quality, quantity and condition of agricultural products...and to conduct information programs designed to eliminate artificial barriers to free movement of agricultural products."

Part of three programs are included in this report: one, a cooperative service program financed with matched funds, carried out by State Departments of Agriculture; the second, a statistical service program of livestock statistics; and the third, provides the marketing services of standards, grades, and market news for the livestock industry. The brief report of selected activities conducted under these programs is included because they are closely related to research work included in the report.

The value of service, educational, and regulatory programs can be observed every day around us. The statistics and other information are used constantly in making business decisions by persons in all segments of the livestock industry from producers to consumers. Inspection, grading and regulatory activities insure a constant supply of wholesome meat and meat products. A classic example of researcheducation-regulatory cooperation is the eradication of the screwworm in the Southeastern United States. These few examples illustrate how cooperative activity can assist in helping all of us to enjoy the high standard of living which American agriculture makes possible.



#### I. FARM RESEARCH

### BEEF CATTLE - BREEDING Animal Husbandry Research Division, ARS

Problem. Expression of each of the productive and carcass traits of beef cattle varies from breed to breed and between animals within each breed. The beef cattle producer is constantly striving to achieve excellence in one or more of these traits. Frequently his failure to choose the best animals for breeding stock or the most effective mating program results in less than maximum progress. Often the beef cattle producer does not know how to identify, evaluate and utilize the existing variability to achieve his aim. Research information is needed on heritability of economic traits in beef cattle, genetic and phenotypic correlation between these traits, effectiveness of various selection and breeding programs, and assessment of traits most useful in beef cattle improvement.

## USDA PROGRAM AND RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

The beef cattle breeding research in the United States has developed as a coordinated program of the USDA and the State experiment stations. It is a continuing program of both applied and basic research carried on by geneticists, animal physiologists, and animal husbandmen. Early efforts in the improvement of beef cattle through performance testing were made by the USDA at Miles City, Montana, and Beltsville, Maryland. With the advent of regional research, efforts by the State stations were greatly increased and the individual programs were coordinated through regional research projects in three of the important beef cattle producing regions. This joint activity has been and remains characteristic of beef cattle breeding research, and the resulting program is an integrated effort combining to the best advantage the resources of the State experiment stations and the USDA.

The regional project in the South is S-10, Improvement of Beef Cattle for the Southern Region through Breeding Methods. Much of this region is subtropical in climate and in many cases cattle used in other areas are poorly adapted. Environmental conditions adversely affecting survival, reproductive regularity and growth are encountered. Research includes projects at 13 State stations and at the USDA stations at Jeanerette, Louisiana; Front Royal, Virginia; and Brooksville, Florida.

In the Western region the beef industry is largely geared to range conditions with many cattle shipped to areas of abundant grain supply for fattening. Ability to make maximum use of forage available on the range is an important consideration. These problems are studied through regional project W-1, The Improvement of Beef Cattle through the Application of Breeding Methods. Research includes projects at 12 State stations and at the USDA station at Miles City, Montana.

Similarly, NC-1, Improvement of Beef Cattle through Breeding Methods, is geared to problems of the beef industry in the North Central region where beef is produced on farms with pastures of high productivity and ample grain supplies for feedlot finishing. Research includes projects at 12 State stations and at the USDA stations at Fort Robinson, Nebraska, and Fort Reno, Oklahoma.

A coordinator for each of these regional projects is provided by the USDA. A number of the States in these three regions have other research in beef cattle breeding which is not formally contributing to the regional program but is closely associated and coordinated with the overall effort. Beef cattle breeding research of limited scope is also conducted at four State stations not in regions with regional projects, and at Beltsville, Maryland.

The <u>Federal</u> scientific effort devoted to research in this area totals 17.4 professional man-years. Of this number, 1.3 are devoted to <u>performance testing</u>, 4.7 to <u>genetics</u> and <u>interrelations</u> of <u>performance traits</u>, 1.0 to <u>genetic environmental interactions</u>, 7.7 to <u>selection and systems of breeding</u>, and 2.7 to <u>program leadership</u>.

State experiment stations in 1961 reported a total of 62.2 professional man-years divided as follows: performance testing 8.4; genetics and interrelationships of performance traits 18.0; and selection and systems of breeding 35.8.

<u>In industry</u> at least one meat packing company has a developmental and demonstrational project on beef cattle improvement. Two breed registry associations have conducted studies on dwarfism. A State cattle feeders' group has assisted in coordinating ranchers, feeders, and packers in providing cattle and services for a study on crossbreeding. These efforts amount to approximately 4 professional man-years.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

Because of the longtime nature of most beef cattle breeding projects it is difficult or impossible to report progress on an annual basis in a completely clear-cut manner. In the material which follows an attempt has been made to discuss those items on which data analysis or summaries during the reporting period have brought out specific information. In some cases it is new. In other cases it supplements previous results.

#### A. Genetics and Interrelationships of Performance Traits

#### 1. Genetic Defects.

Research on hereditary dwarfism was continued during the year on a reduced scale. Further research with radiographs of the lumbar vertebrae show the same accuracy of this technique as reported in

previous years (approximately 80%). In general, a higher percentage of bull calves than heifer calves are predicted as "carriers" on the basis of this technique. (AH dl-31)

Hematological studies in regard to exploring possible techniques for identifying carriers of the dwarf gene have been continued during the year. No new techniques that show promise have evolved from these investigations. (AH dl-31)

At California test matings of Dexter to brachycephalic, dolichocephalic, and comprest mutants produced distinct types discontinuous in size. "Bulldog" calves sired by Dexter and aborted at seven months showed complete fusion of the spheno-occipital synchondrosis. (AH d1-39)

The Florida Station has concluded that the compact animal, known in Florida as the "guinea" is the heterozygote for the Dexter bulldog gene. They have also indicated that there is some genetic or physiological relationship between the guinea and the snorter dwarf since crossing them has resulted in a Dexter bulldog and numerous resorptions. Their work has also indicated that the Brahman carries the snorter gene and the midget is probably the heterozygote for the snorter dwarf gene. The Florida work indicates that mixed breeding may modify the expression of dwarfism. Cooperative work with the medical school at that station on acid mucopolysaccharidosis in dwarfs continues. Carbazole and napthoresorcinal tests for hexuronic acids in the urine will be made on carrier and clean calves. The Texas station is continuing their work on the relationship of amino acid metabolism to mucopolysaccharide accumulation in dwarf carrier and clean animals. (AH dl-9, dl-22, dl-34)

Interpretation on genetic relationships of different types of dwarfism continues to differ with California workers interpreting genetic and anatomical data from specific matings as lending support to the theory that achondroplasia is a complex of a number of components genetically related. On the other hand, results of additional matings at Ft. Reno, Okla., continue to indicate that the "snorter" dwarf, "long-headed" dwarf and the "comprest" condition are each due to different alleles with the "snorter" and "long-headed" dwarf condition being inherited as simple autosomal recessives and the "comprest" condition inherited as an incomplete dominant. (AH dl-39, dl-31)

Results summarized during the year show a "spastic" lethal in Hereford cattle to be inherited as a simple autosomal recessive. "Malocclusion," another achondroplastic condition was reported in Hereford cattle. It is also inherited as a simple autosomal recessive. (AH dl-12)

Previous work had shown hydrocephalus to be inherited as a simple recessive. Some workers now feel that this condition cannot be completely separated from achondroplasia. (AH dl-39)

A sire in one of the Oregon lines that had 7 hydrocephalic calves out of 9 born was subsequently test-mated to 20 females within this same line. These females were divided into groups of 10. The first group was fed iodized salt and the second group got no iodized salt. The first group had one hydrocephalic calf. While the evidence so far is not conclusive, the Oregon workers feel that we cannot yet rule out the possibility that hydrocephalus is caused by a genetic-nutritional interaction involving lack of iodine. Quite possibly there are different types of hydrocephalus. (AH dl-19)

New Mexico has analyzed data and is preparing a manuscript on vaginal prolapse. The tendency of some bulls to sire daughters with a high incidence of vaginal prolapse suggested the trait may be heritable. It was noted that incidence of prolapse differed between the two herds, one run under intensive farm conditions and the other on open range, with the incidence much higher under the farm environment. (AH dl-18)

#### 2. Performance Traits.

Heritability estimates for growth rate from birth to weaning continue to indicate that there are genetic differences between sires. However, information from 1448 calves from 29 Angus, 39 Hereford and 16 Shorthorn sire groups from the Mississippi station indicates that the heritability estimates for growth rate from birth to weaning might be lower than has been previously indicated. When analyzed within breed, these data indicated that heritability for growth rate for Angus was 0.10, Herefords was 0.23 and Shorthorns was 0.12. Heritability estimates of weaning weight were 0.17 for Angus, 0.45 for Hereford and 0.15 for Shorthorn. (AH d1-28)

Data on the progeny of high and low gaining sires from two different stations indicate that the average of the progeny of the two groups for growth rate will be ranked the same way as the sires themselves.

Results continue to show high heritability estimates for carcass weight, rib-eye area and tenderness, while the estimates of heritability of percent untrimmed round, percent major wholesale cuts (untrimmed), percent fat, percent lean of the 9-10-11 rib cut and fat thickness at the 12th rib were somewhat lower. (AH dl-12, dl-38 (c))

Different analytical procedures were compared for obtaining paternal half-sib estimates of heritability. This study demonstrated the necessity for making logical decisions in considering a variable fixed or random in that analytical procedure used appreciably affects the estimate obtained.

In a cooperative study at St. Paul, Minn., attempts are being made to evaluate relationships of dairy and beef characters by improving milk production in a herd of Milking Shorthorns and observe trends in beef

characters of steers produced in the herd as milk production increases. To date average milk production has been only about half (8,000 lbs. vs. 16,000 lbs.) and calves have grown more slowly as compared to a Holstein herd maintained under the same conditions. (AH dl-27)

#### 3. Genetic-Environment Interactions.

As detailed below, quite a few bits of data strongly suggest the reality of genetic-environmental interaction but do not permit estimates of whether they are important enough to make the development of many strains or breeds each adapted to a particular environment, a necessity for maximum production.

At Beltsville, Md., a trial has been completed in which pairs of identical twins were split and one member fed on a high concentrate ration and the other on a high roughage ration. Pair-ration interactions were statistically significant for rate of gain, efficiency of gain and several carcass traits reflecting fat percentage. (AH dl-32)

A three-year test of four inbred lines crossed to grade cattle has been completed at Oregon. Significant differences in year and year x line of sire were observed. However, performance within the lines was in agreement with the performance of the crossline progenies. As a result of these studies, a four-year diallel breeding program involving three of the above inbred lines has been initiated in an attempt to test combining ability and derive new gene combinations containing the desirable traits exhibited in each of the original lines. (AH dl-19)

Nevada's small animal investigations revealed that rats from lines selected for 70-day weight under a high plane of nutrition were not consistently superior for weight at 70 days to rats from lines selected under a low plane of nutrition. Moreover, a high incidence of sterility was observed in the line selected for size under a high plane of nutrition.

In cattle studies at this station, calves within the line selected for conformation tended to gain at a slower rate and were less efficient than calves from lines in which selection was practiced for rapid gain or efficient feed conversion. Within the two locations where cattle are being studied, calves from rate-of-gain and economy-of-gain lines appeared to be similar in performance. (AH dl-36)

Recently, two studies have been initiated in the Southern Region to try to arrive at estimates of the magnitude of this genetic-environmental interaction. A North Carolina experiment is utilizing four locations within the State and different treatments at each location to evaluate how the progeny of sires react to different conditions. They are

utilizing artificial insemination so that the same sires can be used in different treatments and different locations each year. (AH dl-23)

An interregional study has been initiated using cattle from Miles City, Montana, and Brooksville, Florida. Cattle from Miles City have been transferred to Brooksville. Additional transfers will be made this year so as to evaluate how Miles City, Montana, cattle selected for that area respond to selection in Florida and vice versa. This is the first year for this project.

#### B. Performance Testing

Attention to improving methods for evaluating performance in beef cattle is continuous in most projects. The most significant overall recent trends are increased attention to (1) carcass evaluation including methods of estimating carcass characteristics from live animals, and (2) evaluation of fertility and the components or factors upon which it depends. Routine evaluations of these traits will make more comprehensive future genetic analyses possible.

Research involving the use of ultrasonics for measuring differences in fat and muscling in live beef cattle is generally encouraging. Correlations between estimates with this equipment in live cattle and measures in the carcass have been 0.6 to 0.8 for fat thickness and ribeye area. (AH dl-10)

Other studies with other operators have been less promising. At the U. S. Range Livestock Experiment Station, ultrasonic estimates of rib eye and fat depth were made on 52 steers. The partial correlations with live weight held constant were: Ultrasonic rib eye and carcass rib eye, 0.07; ultrasonic fat depth and carcass fat depth, 0.15; and ultrasonic rib eye and fat depth, -.04 (AH d1-2)

Correlations between subjective estimates of fat thickness and rib-eye area in slaughter cattle with measures of these traits in the carcass have averaged approximately 0.4 to 0.5. This was among slaughter cattle of rather uniform weight and condition. Results continue to show that most of the variation in carcass grade can be accounted for by differences in marbling. Cattle involved in these studies ranged from USDA Good to USDA Prime. Studies continue to show a very low correlation (0.1 to 0.2) between thickness of outside fat at 12th rib and marbling score in the USDA Good to USDA Prime grade range. (AH d1-12)

Utah studies show very low correlations between marbling and tenderness. Tenderness has not been related to preslaughter rate of gain. (AH d1-20)

Studies have been initiated involving the use of the Liquid Scintillation Counter for measuring differences in Potassium-40 content of trimmed and untrimmed wholesale cuts. These studies also involve physical

separation of fat, lean, and bone of these cuts, as well as chemical determinations of ether extract and nitrogen.

Studies continue to show variations in fat to be the most important single factor affecting yield of trimmed retail cuts. Studies show only a small amount of variation in distribution of trimmed wholesale cuts. Preliminary results indicate that the yield of trimmed retail cuts from the round, loin, rib and chuck can be predicted rather precisely from a knowledge of fat thickness at 12th rib, rib-eye area, kidney and pelvic fat and carcass weight. (AH dl-12)

The Texas station has derived mathematical equations for estimating the amount of lean in the carcass using carcass weight, percent kidney fat, thickness of fat over the rib-eye and loin-eye area. Equations have also been developed by the Tennessee station using rib fat thickness and carcass weight, as well as other factors. (AH dl-9, dl-22)

Further studies of tritium and N-acetyl 4-aminoantipyrine have shown that they are reasonably accurate for estimating body composition in live cattle. (AH dl-20)

A curvilinear relationship of fat thickness and rib-eye area at 12th rib with carcass weight was found for both heifers and steers.

Up to about 725 pounds carcass weight, heifers had larger rib eyes than steers but the increase in rib-eye area leveled off more rapidly for heifers. For fat thickness the regressions were nearly identical for the two sexes with the heifers being fatter at the 12th rib than steers of the same carcass weight.

While a seasonal plateau was observed in preweaning gains, using average daily gains from birth to weaning to adjust weaning weight for differences in age was found to be almost as accurate as taking cognizance of this plateau in the development of the most appropriate adjustments for weaning weight. (AH dl-12, dl-13)

Selection indexes used to predict net merit for the economical production of beef were compared in theoretical studies. There were (1) weaning weight, post-weaning average daily gain and estimated feed consumption from weaning to 1000 lbs., (2) weaning weight and post-weaning average daily gain and (3) weaning weight alone. Net merit was defined as a function of weaning weight, post-weaning gain and post-weaning feed consumption. Feed consumption to 1000 lbs. was estimated by fitting a curve to individual feeding data. Index (2) was computed to be .73 as effective as (1) and (3) .24 as effective as (1). Considerable progress could be made in the economical production of beef by selecting for weaning weight and post-weaning gain; however, considerable loss in accuracy of identifying superior animals as defined here would be expected when selecting for weaning weight alone. (AH d1-12)

Studies on milk production of beef cattle are being continued at three It appears from these studies that there are breed differences in milk production and that there is a significant relationship between milk production and calf gains. In a study at the Alabama Station, it appears that milk production, per se, is more important than any of the other component parts affecting slaughter grade. Data from this station indicate that heritability of milk production estimated on a within-sire intra-class correlation basis was approximately .33 for Angus and .46 for Herefords. When estimated on a paternal half-sib basis, these estimates were considerably higher. At the Jeanerette station, where the calves were removed from the cow for a 12 to 16 hour period, then weighed, allowed to nurse, and weighed again, the Sindhi and Brangus cows appeared to be highest in milk production. The Angus and Africander-Angus cows were intermediate and the Brahman cows were lowest. There was little difference in milk production of cows nursing straighbred and those nursing crossbred calves. In this study, cows that were five years of age and older consistently gave more milk than three to four year olds. It appears from these studies and other noncontributing projects that milk production in beef cattle is heritable and that progress in increasing milk production could be made by selecting calves with heavier weaning weights. Further work on this aspect will be continued at these and other stations. (AH dl-6, dl-8, d1-29)

Continuing data on creep-feeding in this area indicate that growth rate can be increased from birth to weaning by creep-feeding, depending to a great extent on pasture conditions. In most studies, however, creep-feeding has not been economical in the Southeastern United States. Gain data from the Texas station have indicated that calves averaging 1000 pounds in 365 days or less can be produced if nutritional and management conditions are optimized. The Texas station is also studying feed efficiency. They have indicated that differences in weight, rate of gain and level of feed consumption often make interpretations of feed efficiency data difficult. When daily feed consumption was equalized among calves, they were able to show that average daily gain on test was quite variable, even though initial weight on test was fairly equal. (AH d1-22)

At Wyoming a project has been underway for several years in which semen is collected and frozen from young bulls prior to slaughter. Semen from those with superior carcasses is later used for breeding. Conception rates from semen of bulls slaughtered at 12-14 months has not been good, so the slaughter age is being increased to 16-18 months. (AH d1-25)

Data are continually being collected and analyzed on environmental factors which affect calf weights and gains. Adjustments for sex of calf have recently been estimated at the Alabama station. This study showed that bulls, on the average, were 23 pounds heavier than steers and heifers were 47 pounds lighter than steers. The Mississippi station reports that they found that steers weighed approximately 22 pounds

more at weaning than heifers and that the male calves weighed 4.3 pounds heavier, on the average, at birth than females. A cooperative study by Texas and Virginia revealed that location had a significant influence on performance records of calves from the two States' Beef Cattle Improvement programs. This study was not conclusive, however, in showing whether or not the same growth adjustment factors could be used in the same locations. (AH dl-4, dl-22, dl-28, dl-29)

#### C. Selection and Systems of Breeding

#### 1. Crossbreeding.

During the year, a summary was completed and a regional bulletin published summarizing all crossbreeding results to date in the southern project. Data on over 6000 cattle in 60 different purebred or crossbred combinations contributing information to one or more phases of the study were included. Conclusions of the study were:

Maternal ability as measured by growth rate of calves from birth to weaning was better in the British-Brahman crossbred dams than the straight Brahmans and much better than the average of the British dams. Crosses between British and Brahman types were significantly heavier at birth than the average of the two parental types. Heterosis for birth weight from crosses among British breeds was small and averaged about two percent. With crosses among the British breeds, heterosis for growth rate from birth to about 15 months of age was about four percent. Limited data at one station suggested a slight decrease in growth rate for backcrosses and a slight increase over first crosses for three-breed crosses. First crosses between British and Brahman types showed 11.5 percent heterosis for growth rate from birth to about 15 months of age. Straight British calves grew about seven percent faster than straight Brahmans. Backcrosses to the British type were more effective than backcrosses to the Brahman type in keeping growth rate near the level achieved in the first cross. Carcass traits not directly related to growth rate showed little evidence of heterosis in crosses and usually were near the average of their parents.

In a preliminary analysis of data at Fort Robinson, Nebraska, on heterosis effects involving reciprocal crosses among the Angus, Hereford, and Shorthorn breeds, it was found that the crossbreds grew approximately five percent faster than the straight breds by the same sires. The crossbreds had more outside and kidney fat than the straight breds at the same age; however, there was no difference in carcass grade. Ribeye area was greater in the crosses at the same age. The crossbreds reached puberty at younger ages and lighter weights than the straight breds. (AH d1-12)

There are several comparisons of crossbreeding systems, such as single-crossing, back-crossing, three-breed crossing, rotational crossing and grading up. Limited data indicate that some of the hybrid vigor is lost in a back-crossing program as compared to single crosses when growth rate is considered. This appears to be true also in a continued back-crossing program. (AH dl-3, dl-7)

In contrast to the Fort Robinson data, Louisiana results suggest that age of puberty is largely additive in inheritance with little evidence of heterosis.

#### 2. Inbreeding.

Data from the Front Royal, Virginia, station, where inbreeding is being carried on in several lines and breeds, indicate that inbreeding effects may be more detrimental to females than to males, as far as birth weight and daily gain to weaning are concerned. Data from this station also show a depressing effect of inbreeding on conception rate. (AH dl-4)

Colorado data show a greater amount of heterosis in female than male calves when inbred lines are crossed. Opposite results have been observed in turkeys leading to the theory that inbreeding depression and heterosis may depend to a considerable extent on sex chromosomes with effects being greater in the sex with two of these. The term "homogametic heterosis" has been coined as a name for the theory. (AH dl-16)

In the Oregon inbred lines it appears that selection was ineffective in correcting depression of preweaning gain but was effective with respect to postweaning gain and efficiency. In general, the first trait is lowly heritable, whereas the second has a much higher value. Therefore, selection should be more effective for the latter. (AH dl-19)

Utah data on inbreeding effects are as follows: Increase of 1% in inbreeding of dam decreased weaning weight of calf 1.52 pounds; increase of 1% in inbreeding of calf decreased weaning weight 0.58 pounds. (AH d1-20)

#### 3. General.

In Montana top-crossing merit of station derived stocks is now being determined by loans of high gaining bulls to cooperative ranchers and the return of test steers from (1) these station bulls and (2) rancher-owned bulls. From these tests the value of stocks for commercial calf production can be assessed. (AH dl-17)

Results continue to show some advantage in growth rate of calves sired by bulls from production selected inbred lines in comparison with bulls

produced in breeders herds. These results have been obtained in top cross tests on commercial herds. (AH dl-13)

Top cross tests of four lines developed at Miles City, Montana, on purebred cows in Arizona failed to demonstrate overall superiority but lines 1 and 9 appeared to have merit for the production of feeder animals either at weaning or yearling age. (AH d1-2, d1-14)

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#### BEEF CATTLE - PHYSIOLOGY Animal Husbandry Research Division, ARS

Problem. Research in beef cattle physiology is required for continued improvement in the efficiency of beef production. Investigations should include all the physiological processes involved in growth, fattening and reproduction. Additional information is needed on the physiological responses to the stress of performance at varying levels and under varying environmental conditions. Additional research is also required to take the factual information obtained and apply it towards modification of existing husbandry practices.

#### USDA PROGRAM

This is a continuing program on the causes of reproductive failure in beef cattle and methods of controlling or improving reproductive behavior by hormonal, nutritional or other treatments. It is carried on by physiologists and animal husbandmen at Beltsville, Maryland, and at the Department's Fort Robinson, Nebraska, Miles City, Montana, and Jeanerette, Louisiana, stations in cooperation with the respective State Experiment Stations. Studies on the causes of reproductive failures are conducted with the herds at all these locations. Investigations on the relationship between reproductive performance and protein and energy intake levels are in progress at Beltsville, Fort Robinson, and Jeanerette. Also at Beltsville, studies are in progress on the reproductive performance of cattle exposed to high temperatures and humidity.

Research at Fort Robinson, Nebraska, includes studies on semen evaluation techniques, control of the estrus cycle and the relationship between anatomy of the pelvis and calving difficulties.

The <u>Federal</u> scientific effort devoted to research in this area totals 3.5 professional man-years of which 3.1 are devoted to <u>physiology of</u> reproduction and .4 to program leadership.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State experiment stations in 1961 reported a total of 14.1 professional man-years divided as follows: physiology of reproduction 8.2, environmental physiology 2.3, and physiology of growth and development or other physiology 3.6.

Several stations are conducting investigations on the effect of controlled temperature and hormones on reproduction in heifers of both Brahman and British Breeds, the nature of sterility in animals which leave herds because of failure to reproduce, the fundamental principles

related to ova transfer, and techniques for collection and transfer of ova without surgery. Studies of physiological effects of various hormone substances and developments of simplified methods for bringing groups of animals into estrus within a short period are in progress at other stations. Basic studies are being conducted on the sites of maturization of sperm. Research is being undertaken on biological measures of response to environmental stress under controlled conditions, procedures for measuring environmental responses under field conditions, and the effect of nutrient restriction following weaning on the growth of heifers and upon subsequent lifetime production. Some of the investigations seek to explain the action of hormone-compounds in promoting growth and the effect of levels of milk and forage intake at different periods on gain and weaning weight of beef calves.

There also is research on exposing male and female cattle to varying levels of gamma radiation.

The industrial research in beef cattle physiology is conducted primarily by pharmaceutical companies with some undertaken by certain members of the meat-packing industry. This work is primarily on the use of hormones to promote rapid gains. At least one organization is known to be studying the regulation of estrous cycles with hormones. Approximately 20 professional man-years is estimated as being engaged in this industry activity.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

#### A. Physiology of Reproduction

1. Effects of protein-energy ratios on reproduction have been studied at the Agricultural Research Center, Beltsville, Maryland, and at the Iberia Livestock Experiment Station, Jeanerette, Louisiana. The first phase of this study has been completed at both stations with the following results: Most heifers on the low levels of energy did not reach puberty and this trend was also true for heifers on low levels of protein. However, the heifers on low protein also had a reduced feed intake and were therefore consuming low energy levels of feed. Heifers on low levels of energy and/or protein came in heat and settled when a more adequate ration was fed. Milk production and calf growth were reduced in groups on low levels of energy or protein. Interval from calving to first heat and conception was much longer in the low level cows even after they were raised to an adequate roughage ration. After heifers were placed on a high roughage ration differences in size disappeared and no differences in reproductive performance were noted.

The second phase of this experiment is in progress using 60 grade Angus heifers to investigate the causes of abnormal births and calving losses

in extremely fat heifers and compare three levels of energy and protein which are between the levels which gave the best and poorest reproductive performance in the first phase. Studies include testing the calves to determine the maternal effects on the subsequent growth and performance. (AH d2-22)

- 2. At Fort Robinson, Nebraska, studies were continued on effects of total feed or energy intake on reproductive processes in beef cows. Sixty-nine pregnant Hereford cows weighing an average of 1,111 lb. and having average condition grades of 6.8 in the fall, were wintered to calving on a submaintenance ration of 4.7 lb. of TDN daily. Weights the day after calving averaged 862 lb. and condition scores had gone down to an average of 3.7. Immediately after calving they were allotted to five treatments ranging from 8.5 to 25 lbs. of feed daily. Eightyfour days after calving cow weights by lots ranged from 852 to 1033 lb. and body condition scores from 4.4 to 5.8. The interval from calving to first estrus varied from 49 days for one group to 82 days for another. The percentages of cows conceiving on 1st service were varied from 31 to 87, while the percentages which became pregnant by 120 days after calving ranged from 71 to 100 for different groups. In all cases higher feed levels were associated with best performance. This experiment shows that adequate energy levels are required after calving both for prompt return to heat and optimum conception rates. (AH dl-37)
- In an effort to predict calving difficulty in two-year-old heifers the pelvic area was measured in approximately 1,000 heifers on cooperating ranches. To date results have been tabulated from only two ranches. At Ranch I the incidence of cows having severe calving difficulty varied from 50% for those having pelvic areas less than 170 sq. cm to 11% for those having pelvic areas between 190 and 209 sq. cm. On Ranch II the incidence of severe calving difficulty varied from 49% for heifers having a pelvic area between 180 - 199 sq. cm. to 6% for heifers having a pelvic area between 220 - 239 sq. cm. Birth weights were available at one ranch. Heifers giving birth to calves weighing as much as 70 - 79 lb. had calving difficulty regardless of the size of the pelvic opening. However calving difficulty tended to be less severe as the size of the pelvic opening increased. The only severe calving difficulty encountered in heifers giving birth to calves weighing between 50 and 69 lb. was found in those heifers having pelvic openings less than 190 sq. cm. (AH d1-37)
- 4. In studies on <u>estrual cycle control</u> two hundred cycling heifers received 24 daily injections of progesterone alone or in combination with estradiol (20 mg. of progesterone with 0, 10, 20, or 40 mcg. of estradiol or 40 mg. of progesterone plus 0, 20, 40, or 80 mcg. of estradiol). One group of heifers on each level of progesterone received 20 mcg. of estradiol on the 23rd, 24th and 25th day of injection only. Twenty heifers were not treated and served as controls. Four

heifers showed estrus during the injection period. The proportion of heifers showing estrus during a 5-day period following termination of treatment varied from 68% in the group receiving 40 mg. of progesterone and 20 mcg. of estradiol on 23rd, 24th and 25th days only to 100% in the group receiving daily injections of 40 mg. progesterone in combination with 20 mcg. of estradiol. The heifers conceiving at the synchronized estrus varied from 13% for those receiving 40 mg. of progesterone alone to 56% for those receiving 40 mg. of progesterone plus 40 mcg. of estradiol. Sixty percent of the controls conceived on first service. (AH d1-37)

- 5. Age and weight at puberty were determined on 40 straightbred and 47 crossbred heifer calves. These represented progeny from Angus, Hereford and Shorthorn sires bred to females of their own breed and to females of each of the other two breeds. The crossbred heifers attained puberty 58 days earlier and were 27 lb. lighter than the average of the straightbreds. The difference in age at puberty was reduced to 20 days when adjusted for both 200-day weight and average daily gain from 200-396 days. Increased growth rate of the crossbred heifers appeared to account for about two-thirds of the difference in age at puberty. Straightbred Angus, Hereford and Shorthorn calves reached puberty at 382, 483, and 427 days and weighed 524, 615, and 510 lb., respectively. Differences between reciprocal crosses were negligible. Important differences in age and weight at puberty were shown among sire groups. (AH d1-12, d1-37)
- 6. Results from the 1961 breeding season at Fort Robinson show that cows being bred for crossbred calves had superior reproductive performance. The percent pregnant was 97% for cows being bred for crossbred calves; 91% for cows being bred for straightbred calves. Cows bred for straightbred calves took an average of 1.94 services per conception and 40% settled on first service, while cows bred for crossbred calves required 1.78 services per conception and 52% settled on first service. (AH d1-12, d1-37)
- 7. In efforts to find <u>factors related to stillbirths</u>, data were obtained over a five year period at Miles City, Montana, on blood plasma levels of carotene, vitamin A, and inorganic phosphorus in cows giving birth to stillborn calves. Detailed postmortems were made on most of 154 stillborn calves and 22 calves that died at less than seven days of age.

More male than female (83 vs. 51) calves were stillborn and the stillborn males averaged 20 lb. heavier than stillborn females. More males exhibited abnormal presentations and required birth assistance. More males showed postmortem symptoms of injury due to prolonged parturition.

Three-year-old dams had the highest incidence of stillborn calves followed by four-year-olds.

Other than symptoms of difficult birth, the most frequent clinical finding in stillborn calves was heart abnormalities including patent auricular, ventricular or auricular-ventricular systems.

Calf deaths within one week were largely due to disease with pneumonia being the most frequent.

Mean blood plasma values of cows giving birth to stillborn calves were within the normal ranges in carotene, vitamin A and inorganic phosphorus. (AH dl-33)

8. In an effort to determine <u>supplements needed on pasture for optimum reproduction</u>, an experiment was started at Jeanerette, Louisiana, in November, 1961, with pregnant, coming 2-year-old Angus heifers. Treatments are: Lot I, pasture only; Lot II, dry lot, fed to maintain same weights as Lot I; III dry lot, fed according to National Research Council recommendations for pregnant and lactating heifers; and Lot IV, pasture with supplemental feed as needed to maintain same weights as Lot III. The study thus provides for studying reproductive performance at two levels of nutrition, in dry lot and on pasture at the same nutritive levels, and will provide two estimates of the amount of nutrients actually obtained at different seasons from mixed white clover-grass pasture at that location.

Initial weights averaged 712 lb. By June 1, 1962, Lots I and II averaged 70 lb. lighter than Lots III and IV and only 19% had been in heat as compared to 80%. The results indicate the inadequacy of pasture alone at this location for optimum reproductive performance by young cows. (AH d2-34)

9. In studies of <u>factors affecting herd reproductive rates</u>, breeding herds at Jeanerette, Louisiana, consisting of purebred Angus and Brahmans strains based on <u>inter se</u> matings of Angus x Brahman and Angus x Africander foundations and Angus-Brahman lst crosses were routinely checked for puberty, interval from calving to first estrus, and conception rate during a 75 day breeding season.

Angus heifers were youngest at puberty, Brahmans the oldest and the other three strains intermediate. Post partum intervals to estrus were longer for three-year-old cows than for older animals in all breeds and Brahman, Brahman-Angus, and Africander-Angus cows had longer intervals than Angus. Brahman cows had a lower conception rate than other types.

Heifers and cows not becoming pregnant in the regular breeding season were further investigated. A group of the open cows were placed with a fertile bull in September while lactating. Only twenty percent of the cows showing heat settled during this period. After the calves were

weaned, 50% of the cows showing heat, conceived. At slaughter, regressing embryos and membranes were found in a number of the non-pregnant cows indicating embryonic death had occurred. Abnormalities found included: (1) infantile reproductive organs; (2) double cervix; (3) lutenized follicles; (4) blocked oviducts and (5) ovarian adhesions. (AH d1-6, d1-30)

10. In experiments on temperature effects on reproduction in heifers previous work had shown that heifers placed in a heat chamber at 900 F. during the winter went through a period of heat stress but eventually shed their winter coats, resumed estrual cycles, and were fertile. To check this, six Hereford heifers were placed on experiment in December for 16 weeks. They were higher in condition and had a shorter hair coat depth than the heifers in the first trial. Hair coat depth increased slightly until the third week then remained at this level until the ninth week when the hair coat decreased. Rectal temperature and respiration rate increased markedly up to the third week then remained at approximately this level while experiencing numerous peaks and dips. In this test as stress built up the heifers would leave their feed until their temperature would drop and then start eating their feed again. During the experimental period four heifers ceased to cycle but did reestablish their estrous cycles by the 12th week.

In an effort to determine whether previous conditioning would affect responses, three groups of heifers were placed on experiment in September at Beltsville, Maryland, after a summer grazing period. One group was kept in a psychrometric chamber for 14 weeks at 90° F. The other groups were kept under prevailing ambient temperature conditions with one group having artificial light similar to that of the heat chamber while the other had natural light. The groups housed under ambient conditions showed no significant changes in respiration rate or rectal temperature but water intake followed seasonal temperature while the chamber group showed significant changes in these measurements. Hair coat depth increased slightly up to the fourth week then remained at this level for the rest of the period. Only one animal ceased to cycle during the experimental period. Indications are that a rise in rectal temperature had less effect on estrous cycle in summer-conditioned heifers.

To further test the effects of high temperature on the estrous cycle, six heifers were allowed to become conditioned to summer conditions. They were then placed in the chamber in mid-August for an 8 week period at  $100^{\circ}$  F. Body temperatures rose to very high levels and after a small drop remained at a high level for the test period. During the test period, all but one heifer ceased to cycle. This would indicate that the estrous cycle can be stopped if the heat stress is severe enough. (AH dl-30). (Also reported in Area 6)

#### PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

#### Physiology of Reproduction

- Wiltbank, J. N., Rowden, W. W., Ingalls, J. E., Gregory, K. E., and Koch, R. M. 1962. Effect of energy level on reproductive phenomena of mature Hereford cows. Jour. Anim. Sci., 21(2), pp. 219-225.
- Wiltbank, J. N., Rothlisberger, J. A., and Zimmerman, D. R. 1961. Effect of human chorionic gonadatrophin on maintenance of the corpus luteum and ambryonic survival in the cow. Jour. Anim. Sci., 20(4), pp. 827-829.
- Wiltbank, J. N., Warwick, E. J., Vernon, E. H., and Priode, B. M. 1961. Factors affecting net calf crop in beef cattle. Jour. Anim. Sci., 20(3), pp. 409-415.
- Wiltbank, J. N., Ingalls, J. E., and Rowden, W. W. 1961. Effect of various forms and levels of estrogens alone or in combinations with gonadatrophins on the estrous cycle of beef heifers. Jour. Anim. Sci., 20(2), pp. 341-346.

# BEEF CATTLE - NUTRITION AND MANAGEMENT Animal Husbandry Research Division, ARS

Problem. Producers of beef cattle need improved feeding methods which will result in optimum pasture gains, reduced feed consumption per pound of beef produced, optimum reproductive rates and desired carcass qualities. To meet these needs much basic nutritional information is required, such as: When should beef animals be fed for maximum gains and when for more limited gains? What nutrient combinations produce rapid growth of muscle with a minimum of fat deposition? How may breeding animals be economically raised that will be capable of a high level of reproductive performance over a long lifetime? What are the nutritive contributions made by range and pasture and what supplementation is required when they are used? Research is also needed on the relation between animal production and types of shelters and equipment, feeding systems, and methods of increasing labor efficiency.

#### USDA PROGRAM

This is a continuing program carried on by nutritionists, biochemists and animal husbandmen on basic and applied problems related to feeding and management of cattle for beef. The work is in progress at Beltsville, Maryland; in cooperation with State experiment stations at federally—owned stations in Miles City, Montana; Fort Robinson, Nebraska; Ft. Reno, Oklahoma; Jeanerette, Louisiana; Brooksville, Florida; and Front Royal, Virginia; and in cooperation with State experiment stations at the State locations of Raleigh, North Carolina; Tifton, Georgia; College Station, Texas; and Newell, South Dakota.

The <u>Federal</u> scientific effort devoted to research in this area totals 10.7 professional man-years. Of this number 2.7 are devoted to <u>digestion and metabolism</u>, .6 to <u>concentrates</u>, 2.7 to <u>forage preservation</u> and <u>utilization</u>, 1.4 to <u>nutrient requirements</u>, 1.0 to <u>range and pasture</u> management, .7 to management practices, and 1.2 to program leadership.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State experiment stations in 1961 reported a total of 106.8 professional man-years in this area of which 15.3 were devoted to digestion and metabolism, 18.2 to concentrates, 26.8 to forages, 19.7 to nutrient requirements, 16.2 to range and pasture management, and 10.6 to management practices.

In the area of digestion and metabolism, the primary research is on the basic functions of the rumen, particularly the animal feed interrelationships which are responsible for bloat, efficient feed digestion, and synthesis of essential nutrients.

The ways in which concentrate feeding can be modified to improve beef cattle production efficiency are under study as follows: (1) The response of breeding or fattening animals to various forms of concentrate supplementation, (2) The value of creep-feeding, (3) The effect upon gain and feed efficiency of frequent feeding compared with feeding once or twice a day, and (4) The use of salt or gypsum to limit feed intake.

The effect upon nutritive value of various methods of harvesting, storing, and feeding concentrates are also under study.

In the forage area, investigations are underway on the effect of fertilization, time of cutting, method of storage, and methods of feeding upon the nutritive value of forages are of major concern. The development of methods for the accurate evaluation of grazed forages is under study as is the stimulation of forage digestion by ration supplementation with hormones, enzymes, antibiotics, and minerals. Several stations are cooperating in regional projects on problems in this general area.

The basic requirements of beef cattle for specific nutrients, their metabolism, interactions, and availability in feeds, constitutes a major area of work in beef cattle nutrition. Studies are underway in the following general areas: (1) The requirements, metabolism, and interactions of the many major and trace nutrients, (2) The effect of feed additives or implants upon growth and feed efficiency, (3) The relation of nutrients to metabolic disorders, such as urinary calculi and grass tetany, (4) The toxicity of molybdenum and fluorine, (5) The value of irradiated feeds in assimilation of fallout products, (6) The use of roughage concentrate ratios in chemical regulators for feed intake control, and (7) The effect of physical form of the ration upon nutritional value.

Work is underway on the evaluation of the composition of range and pasture plants, the quantity harvested by livestock, the use of pasture or range by various ages of cattle, and the effects of stocking rate upon the output of beef per acre and individual animal performance.

Studies on management practices, equipment, and facilities, include such things as various combinations of drylot and pasture feeding, maximum utilization of pasture through using late fall and early spring pasture crops and winter pastures in the South, use of supplementation necessary when low quality roughages are used by breeding animals, and creepfeeding vs. noncreep-feeding of calves.

Approximately 60 professional men are engaged by <u>industry</u> and <u>other</u> <u>organizations</u> in the field of beef cattle nutrition and management. The greatest portion of this work is on feed additives, supplement formulation, and comparisons of rations. Some of the initial work on all concentrate rations was conducted in the feed manufacturing industry. At least one agricultural chemical company is studying the metabolic

disorders resulting from fluorosis. There is one private research foundation engaged in studies in nutrition with beef cattle.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

# A. <u>Digestion and Metabolism</u>

### 1. Digestion Techniques.

Digestibility techniques are being studied at Raleigh, North Carolina, and Beltsville, Maryland. Data collected since 1957 on application of the chromic oxide method for estimating digestibility of complete, mixed, and pelleted rations are being summarized. Statistical estimates of error of prediction will be calculated. If the errors are within an acceptable range, the technique will be used in making routine digestibility observations which often accompany feeding trial data. The chromic oxide method has certain advantages over other indicators, such as lignin and chromagen.

Much of the recent work has been devoted to the study of certain polyphenols which might influence digestibility of sericea lespedeza. A potent enzyme inhibitor has been isolated which, in vitro at least, inhibits cellulases, pectinases, pectinesterases and amylases. This polyphenol has properties which are usually attributed to leucoanthocyanins. It gives positive tests to vanillin-HCl and HCl. The inhibitor has not been isolated in large enough amounts so that in vivo studies can be made. Inhibition of cellulose digestion has been demonstrated both in cellophane tubing in a steer with a rumen fistula and in an artificial rumen.

The inhibitor is either destroyed or changed in properties in the dehydration process. Tests have been made which show that the inhibitor is changed before it reaches the dehydrator. Samples which were taken directly from the field chopper indicated that the inhibitor is lost at this point. The loss from bruising or chopping is possibly due to oxidation, either by atmospheric oxygen or possibly by polyphenol oxidases.

Work was continued in the prediction of digestibility of forages from chemical composition. The protein of sericea does not follow the usual prediction equation for protein. Reasons for these discrepancies will be studied further. (AH d2-8, d2-14)

#### 2. Feedlot and Pasture Bloat in Beef Cattle.

Research on feedlot bloat in beef cattle at Beltsville has been directed toward determining the importance in relation to bloat of the diluting effect of saliva on ruminal contents and of the inorganic salts present in saliva. Increased water intake and the consumption of synthetic salivary salts by cattle did not significantly influence the incidence of feedlot bloat. The pH of the ruminal samples was higher when synthetic saliva salts were fed. Neither surface tension nor total concentration of ruminal fatty acids nor the proportion of acids were affected by treatments. Between animals there was a positive correlation between the concentration of fatty acids in the rumen and bloat. However, within animals the correlation was negative. In addition, the molar percentage of acetic acid salts in the rumen was positively correlated with bloat. There was no correlation between microbial activity of the ruminal samples and bloat.

In a continuation of studies on the effects of vegetable or mineral oil on feedlot bloat, a high incidence of bloat resulted when steers were fed a basal bloat producing ration alone or with eight percent soybean oil added. An eight percent level of mineral oil added to the ration prevented bloat entirely. Data from chemical and physical analyses of the ruminal samples taken during this experiment have not been summarized at this time.

Drenching with soybean oil at a level equivalent to eight percent of the ration tended to decrease feedlot bloat. Further, it caused an increase in the proportion of propionic acid in the ruminal fluid and a decrease in the ingesta volume increase in incubation. Earlier results on the feeding of the same amount of soybean oil mixed into the entire ration indicated an increased incidence of bloat with the addition of soybean oil with no effect on the ruminal characteristics.

Mixing mineral oil in a bloat-producing ration at levels of 0.1, 2, and 4% slightly reduced bloat at the 1 and 2% levels and markedly reduced it at the 4% level. The reduction in bloat from the addition of 4% mineral oil was similar in degree to that which resulted from the addition of 8% mineral oil in another experiment reported earlier. (AH d2-13)

# 3. Cause and Prevention of Urinary Calculi.

Basic and applied studies of the cause and prevention of urinary calculi were continued at College Station, Texas, with lambs and initiated at Big Spring, Texas, with steers and lambs. Lambs are used partly as a laboratory animal for beef cattle research and partly to obtain usable information on lambs themselves. Data obtained from experiments conducted in 1958-1959, 1959-1960, and 1960-1961 were summarized and grouped together in an attempt to understand the observed variation in the occurrence of urolithiasis from year to year.

The phosphate type of calculi observed in these experiments with fattening lambs was related to an imbalance in the dietary intake of calcium, phosphorus, and potassium. Animals receiving diets high in

phosphorus and low in calcium and potassium readily developed urinary calculi while diets low in phosphorus and high in calcium and potassium were not calculogenic. The variation in the occurrence of urinary calculi over a three-year period could be explained by differences in the dietary mineral intake of calcium, phosphorus, and potassium. The results of the experiment conducted in 1961-1962 are being analyzed at present and appear to confirm the previous observations.

Results obtained from analyses of the blood and urine samples taken during the experiments indicated that changes in the dietary mineral balance significantly altered the mineral levels of the serum and urine. Lambs receiving excessive phosphorus in their diet excreted <u>increased</u> amounts of urinary phosphorus but <u>decreased</u> amounts of urinary magnesium and these conditions were predisposing to urinary calculi formation. Animals receiving the high-calcium diets had <u>lower</u> levels of urinary phosphorus but <u>increased</u> levels of urinary magnesium. Urinary magnesium was also <u>increased</u> by increasing the potassium in the diet. Serum levels of phosphorus and magnesium were related to the excretion rates of these minerals.

The results of these studies indicate that an excess of dietary phosphorus above the animal's requirement is conducive to the development of urinary calculi unless adequate dietary levels of calcium and potassium are present.

Other factors are, however, also related to calculi formation. Pelleting appears to increase calculi cases with  $\frac{1}{4}$ -inch pellets having a greater effect than 3/8-inch ones. Form of calcium supplement may also be a factor with oyster shell having given a higher incidence of calculi than carbotex.

The combined steer and wether lamb study at Big Spring is not sufficiently advanced because of the lack of numbers to warrant any definite conclusions or trends. Based on the animals examined at slaughter, neither carbotex nor disodium phosphate had any effect on calculi formation in the steer while ammonium chloride sharply decreased stone formation. Insofar as the wether lamb was concerned, disodium phosphate did not increase the percentage of lambs developing calculi. Carbotex reduced the incidence 50% while ammonium chloride effected a 90% reduction over the control or basic ration. (AH d2-31)

# 4. Pesticide Residues.

Investigations of residues left by pesticides which are ingested by beef cattle were started in Beltsville, Maryland, and Tifton, Georgia. Diazinon was added to alfalfa meal and the mixture was pelleted. The pellets were fed to steers in metabolism stalls. Urine and feces were collected and frozen for analysis. No diazinon was lost

during the pelleting process but it was lost from the pellets during storage. The rate of loss is about the same as the rate of loss of carotene from alfalfa pellets. Considerable amounts of diazinon were found in the feces. However, it is demonstrated that oven drying of feces drove off diazinon so that amounts found in the feces were low. A different procedure for this determination will have to be used in future studies.

Residue studies with a pesticide, Thiodan (6, 7, 8, 9, 10-hexachloro-1, 5, 5a, 6, 9, 9a hexahydro-6, 9 methano-2, 4, 3 benzodio-xanthiepin-5oxide), were conducted to determine the stability of the compound in mixed feeds, the physiological effects on cattle when ingested in the feed, and tissue residues and resulting metabolic breakdown products. Thiodan was added as the dry compound to the rations by careful premixing of the chemical with ground corn. Pelleting an alfalfa meal ration (1900 lbs. alfalfa meal, 100 lbs. corn) did not reduce the level of Thiodan when added at a level of 10 p.p.m. A 10% loss of Thiodan occurred when the mixed ration was stored for 30 days. Steers were fed rations containing 10, 60, 100, and 200 p.p.m. of Thiodan or .15, 1.25, 2.5, and 5.0 mg. per kg. of body weight, respectively. No tissue residues of Thiodan were found in omental fat samples of 10 grams, obtained by biopsy, from steers receiving Thiodan at levels of .15 and 1.25 mg. per kg. of body weight for 30 and 14 days, respectively.

Animals fed the pesticide at a level of 5 mg. per kg. of body weight for 2 days or 2.5 mg. per kg. of body weight for 13 days developed symptoms of convulsions exhibited in the muscles of the shoulder and flank. Also, excessive salivation, sweating, and incoordination in walking were observed. These symptoms disappeared within 2 hours after removal of the feed.

The excretion of Thiodan in the feces was extremely variable. Procedures for the determination of possible oxidation products of the pesticide in body tissues, feces, and urine are being developed.

A modification of the colorimetric method for Thiodan residues as developed by the Niagara Chemical Division, Food Machinery and Chemical Division, Middleport, New York, was used for assaying for Thiodan in the feed and animal fat. The method appears to be sensitive to 1 p.p.m. (AH d2-32)

### 5. Microbiology of the Rumen.

Biological and chemical studies of microorganisms in the rumens of beef cattle are being conducted in order to increase understanding of the basic digestive processes which occur in grazing animals. The bacteria and protozoa found in the rumen are involved in the degradation of the digestive foodstuffs and forages. In the normal rumen, fermentation food materials are metabolized to microbial cell protein,

volatile fatty acids and gases. Abnormal rumen fermentations which ofttime result in bloat when a high percentage of grain is fed to beef cattle are characterized by the production of large amounts of slimy materials in the rumen. The slime produced by the bacteria increases the viscosity of the rumen fluid and may serve to entrap the fermented gases in a froth which blocks a normal gas eructation mechanism. Chemical analysis of the slime which was isolated by ethanol precipitation procedures indicates a composition of protein, carbohydrate, phosphorus and nucleic acids. The viscosity of the slime suspension could be lowered by acid or mild heat treatment indicating nucleic acid of microbial origin.

Microbiological studies have also been conducted on the biochemical activities of the protozoa found in the rumen of cattle. The rumen protozoan Ophryoscolex caudatus has been shown to ferment starch with the production of acetic, butyric and lactic acids plus carbon dioxide and hydrogen. Cellulose was not significantly metabolized although pectin was rapidly utilized. Plant protein sources, cottonseed, soybean and linseed meals and the C-14 labelled amino acids alanine, valine, and leucine were metabolized by the protozoan and ammonia was demonstrated as the end product of nitrogenous metabolism. on the biochemistry of the ruminal protozoa are being continued in order to gain additional knowledge of the physiological function of the microbes in the rumen of cattle. It has already been established that the ruminal protozoa aid in the breakdown of plant materials such as starch and cellulose, produce fatty acids which are absorbed and used as a source of energy by the host, and that the cell bodies of the protozoa serve as a source of protein for the host. This type of information will be useful in establishing the minimal nutritional requirements of the microorganisms and thus yield practical knowledge of the nutrition of the host. (AH d2-24, d2-26)

#### 6. Value of High-Nitrogen Molasses.

The high-nitrogen molasses studied is a byproduct of a new sugar-refining process which utilizes the ion exchange principle and results in more economical sugar production. The nitrogen is present in the molasses as ammonium salts of sulfur, phosphorus and carbon. The nutritive value of this molasses for beef cattle was studied in a palatability-toxicity test, a feeding trial and a metabolism trial.

The molasses appeared to be palatable when offered ad libitum to 8 steers on a chopped hay diet during an 84-day test period. An average of 6.4 lb. molasses and 12.5 lb. of hay was consumed per steer per day with no apparent ill effects to the nervous or digestive systems.

During a 168-day group feeding trial, steers performed similarly whether they were fed a ration containing 30% hi-N-molasses or 30% regular molasses plus urea.

There were no differences in the digestibility of the nutrients with the exception of crude protein, which was less digestible (P<.01) in the ration containing the hi-N-molasses. Estimates of digestibility determined in the feedlot using the chromic oxide method confirmed the trends observed using metabolism crates.

There were no treatment differences in nitrogen retention when expressed as q./day or as a percent of the nitrogen consumed.

Metabolism studies with rations containing fifty percent hi-N-molasses have been delayed because of a consumption problem. It has yet to be determined if it is a palatability or nitrogen availability problem. (AH d2-14)

# 7. Coumestrol in Alfalfa.

In cooperation with the Western Regional Research Laboratory, an experiment was conducted at Beltsville to compare digestibility and nitrogen utilization of high- and low-coumestrol alfalfa when fed to beef cattle. The two alfalfa meals differed in quality as indicated by a higher digestible dry matter and crude protein for the low-coumestrol alfalfa. This difference was apparently due to the fact that the low-coumestrol alfalfa was harvested at an earlier stage of growth than the high-coumestrol alfalfa. There was no apparent difference in nitrogen retention when cattle were fed the two alfalfas. (AH d2-8)

# 8. Anatomical and Physiological Factors Affecting Digestibility.

There is relatively little information regarding the independent contributions of the gastro intestinal tract prior to and following the abomasum to digestion. Surgical removal of the rumen has been frequently attempted, but has met with only limited success. Currently, calves are being fistulated at the abomasum and all of their dry matter in pelleted form other than that in milk is being administered per abomasum. It is hoped that information leading to a greater understanding of ruminant digestive physiology will evolve from these studies.

Studies are also in progress to determine some of the rumen factors which influence the rate of salivary secretion. Sheep are being used as pilot animals while steers will be used in the final studies. Salivary collections are by means of esophageal cannulae. Thus far only pelleted rations have been studied. (AH d2-8)

# B. Concentrates

### 1. Feeding Value of Pelleted Feeds.

The feeding value of pelleted feed is being studied at several locations. At Tifton, Georgia, pelleted Coastal Bermuda grass hay and cottonseed meal and baled Coastal Bermuda grass hay and cottonseed meal were compared using cow and calf performance as a measure of efficiency of feed utilization. The cows ate an average of 29.5 pounds of pellets as compared to 25 pounds of baled hay per day. Pelleting the hay resulted in an increase in consumption of about 30%. Both groups ate  $1\frac{1}{2}$  pounds of cottonseed meal daily. The cows eating pellets lost less weight than those eating baled hay, 66 pounds and 17 pounds, respectively. The average birth weights of calves were lighter for the cows full fed hay, 69 pounds compared to 77 pounds for calves from the pellet-fed cows. The daily gains of calves from cows on hay and pellets were 1.68 and 1.86 pounds, respectively, to about 50 days of age.

Long yearling steers weighing about 730 pounds were used to compare Coastal Bermuda grass pellets with and without concentrate with a standard control ration. Relatively good gains were made by steers fed pellets alone for 154 days or pellets plus a short feed of concentrates, but yields and carcass grades were not as high as steers in the control lot fed Coastal Bermuda hay, ground snapped corn and cotton-seed meal. Steers fed only pellets until they reached the slaughter weight of the control group required 203 days. Carcasses from steers fed pellets for 203 days graded approximately the same as the control lot which received the standard fattening ration for 154 days. The results of this test suggest that relatively mature steers can reach a desirable slaughter finish when fed Coastal Bermuda pellets only if fed for a longer time than required on conventional rations.

Compared to 100% Coastal Bermuda grass pellets, the addition of shelled corn either in a pelleted mixture, mixed with Coastal Bermuda pellets or fed free choice with pellets, had no consistent effect on feed consumption, but generally increased gain, dressing percentage, and carcass grade. It was concluded that adding shelled corn to Coastal Bermuda grass either in a pelleted mixture or as a supplement to an all Coastal Bermuda grass pellet would be a desirable practice if this type of ration is to be used for finishing steers for slaughter. Feeding pellets consisting of 95% Coastal Bermuda grass and 5% molasses resulted in increased feed consumption but decreased gain.

Coastal Bermuda grass pellets were compared with a standard fattening ration consisting of ground snapped corn, cottonseed meal, and Coastal Bermuda hay using yearling steers weighing about 760 pounds. All lots of steers showed a higher rate of gain during the first half of the experiment than during the last half. Steers fed Coastal Bermuda

pellets gained more than 2 pounds per day during the first 77 days. Steers fed pellets appeared to be slightly more efficient in conversion of feed to gain. Steers fed the control had higher dressing percentages and higher carcass grades than the steers fed pellets and concentrate and pellets only. The steers on the control ration brought a higher selling price.

At Beltsville, Maryland, a ration containing 60% Bermuda grass hay in ground, heated  $(260^{\circ}\ F.)$  and pelleted forms was compared to a 60% ground alfalfa ration when fed to yearling Hereford beef cattle. All rations contained 36% corn and 4% molasses. The animals were group fed. Steers fed the alfalfa-corn ration made greater gains (ADG = 2.47lb.) than the steers on the Bermuda grass ration. The steers fed the chopped and pelleted Bermuda grass-corn rations outgained (ADG = 1.89 and 2.00 lb.) the steers on the heated ration (ADG = 0.29 lb.). Steers fed the heated ration lost 1.37 lb./day until the ration was supplemented with protein. Steers fed the pelleted Bermuda grass-corn ration required no more feed per pound of gain than the steers consuming the alfalfa-corn ration.

Carcass data from the above experiment and an earlier trial indicated that steers fed pelleted rations tended to have heavier reticulo-rumens when the weight of the organ was expressed as a percent of the weight of the gastro-intestinal tract.

Steers fed rations which had been pelleted exhibited greater molar percentages of butyric plus higher acids in their rumen liquor than steers fed the same ration which had not been pelleted.

Physical state - animal behavior studies in which steers were fed rations ground or pelleted and high or low in roughage content indicated that animals spend less time at the feeder when fed a ration in pelleted form. It was also noted (as was expected) that animals spent less time at the feeder when a high concentrate ration was fed as compared to when a ration high in roughage was offered. Conversely, animals consuming high concentrate rations or pelleted rations visited the feeder more frequently than when they were consuming high roughage or ground rations.

There were significant differences among animals and among periods in respect to the average time spent at the feeder each day. On the other hand, the average number of visits to the feeder each day was similar from period to period. Animals differed in the average number of times they visited the feeder each day.

Replication of this experiment with fourteen-day observation periods, instead of seven, gave similar results. Furthermore, there was no difference in the results when the first seven days of observations were compared with the second seven days—thus suggesting that a seven-day observation period is adequate.

An experiment designed to test the effects of animal size upon feeding behavior is now being completed. Animals ranging from 500 to 1200 pounds in body weight are being used.

In future experiments, such factors as competition, selection, and water availability will be studied. (AH d2-28)

# 2. Keeping Quality of Corn.

The nutritive value of stored corn was compared with new corn in studies at Wooster, Ohio. Shelled corn grown in 1954 and stored under the Federal government loan program was found equal in feeding value to 1960 corn in a cattle feeding experiment completed recently at the Ohio Agricultural Experiment Station.

The results obtained showed no significant difference in feeding value between the ages of shelled corn with respect to average daily gains, feed requirements per unit of gain, carcass grades and dressing percentages. Moreover, both kinds of corn gave about the same results in daily gain and carcass grades when supplemented with Vitamin A.

The Ohio test supplies a provisional answer to livestock producers who have wondered about the feeding value of the millions of bushels of corn held in storage for several years by the Commodity Credit Corporation and now being offered for sale under the 1961 feed grain program.

The test was begun at Wooster on July 18, 1961, with 40 head of yearling steers in drylot and was concluded on October 10. One-half of the steers were fed on 1954 corn and the other half on 1960 corn. Both lots of 20 head each were further subdivided with one-half in each case receiving no Vitamin A supplement while the other half got 20,000 International Units of supplemental vitamin A per head daily.

The corn was ground in a hammer mill and samples of ground corn passed through standard mesh screens showed no difference between the 1954 and 1960 corn in fineness of grind. Corn was hand full fed with good quality mixed hay, soybean oil meal, salt and minerals.

Blood plasma vitamin A levels were determined at the beginning and end of the experiment. Although the feeding of vitamin A brought about a definite increase in blood plasma level, none of the steers were considered to be deficient in this vitamin.

This one short term experiment with only two lots of corn does not answer the question of feeding value for all stored corn nor does it answer the question for other classes of livestock. However, it does show no deterioration in feeding value for fattening cattle on shelled corn which had been in storage for nearly seven years. (AH d2-33)

# 3. Value of LPC Dried Beet Pulp in Fattening Rations.

In studies at Fort Robinson, Nebraska, one hundred and thirty yearling heifers were used to study the effect of changing the percent of LPC dried beet pulp in the ration during the fattening period.

One group was fed 50% cracked corn and 50% beet pulp. The second group was fed 80% cracked corn and 20% beet pulp. The third group was fed 50% cracked corn and 50% beet pulp for the first 28 days then changed to 55% cracked corn and 45% beet pulp. Their ration was then changed every two weeks by decreasing the beet pulp 5% and increasing the corn 5% until the ration was 80% cracked corn and 20% beet pulp. All groups were self fed after the first 28 days and had free access to alfalfa hay and a mineral supplement.

The average daily gain and cost per 100 pounds of gain for groups 1, 2, and 3 were 2.54 pounds, \$17.24; 2.16 pounds, \$18.31; and 2.36 pounds, \$17.85, respectively. (AH d2-21)

# C. Forage Preservation and Utilization

# 1. Utilization of Coastal Bermuda Grass.

The performance of steers grazing Coastal Bermuda grass was measured in a series of experiments extending over a period of two years at Tifton, Georgia. The grazing levels were 1.5, 2.0, and 2.5 steers per acre. The steers made the best gains on pastures which were stocked at 1.5 steers per acre level. The average daily gain decreased as the stocking rate increased. However, gain per acre increased as the stocking rate increased. The increase in gain per acre between the 2.0 and 2.5 level was small. The gain per acre and average daily gain at the three levels were 337, 1.46; 362, 1.18; and 371, 0.96, respectively. Animal performance was poor and the pasture was overgrazed at the 2.5 steers per acre level. The 2.0 steer per acre pastures were slightly overgrazed.

At Tifton, Georgia, a digestion trial showed that 3-week-old Bermuda grass was not very different from 5-week-old grass. The digestible dry matter and TDN were 57.7 and 57.0% for the 3-week-old grass and 56.4 and 56.0% for the 5-week-old grass, respectively. (AH d2-3)

### 2. Nutritive Value of High Moisture Grain Silage.

The protein nutritive value of high-moisture grain stored in silos was measured in studies at Beltsville in cooperation with the Agricultural Engineering Research Branch.

Changes in grain harvesting methods, including the use of mechanical corn pickers and field shelling, have increased the volume of high

moisture corn. This material must be dried before storage or an alternative method is storage in silos. Little work has been reported on the effects on protein nutritive value of high moisture grain stored in silos. Eight samples of high moisture corn were taken from silos on four farms located in the Corn Belt. The samples represented different types of silos and grain with different gross appearance from different areas of the silos.

A routine rat growth method was used to estimate protein nutritive value of the samples. Statistically significant differences were found in the stored samples. Damage to protein nutritive value was found to be associated with dark brown discoloration and strong fermentation odor of the grain. (AH d2-30)

### D. Range and Pasture Management

### 1. Range Supplementation Studies.

A project designed to determine the effect of age at first calving and level of winter feeding of beef cows on the breeding efficiency, longevity, and economic production of calves has been underway since 1948 at Fort Reno, Oklahoma, in cooperation with the Oklahoma Agricultural Experiment Station.

Of the 30 cows starting the test in the fall of 1948 on each feeding level, 16, 11, and 5 remain on the low, medium, and high levels, respectively, as of March, 1962.

The data from the original group of cows have shown that limited amounts of supplement (one pound of cottonseed meal per head) on dry weathered native grass pastures resulted in nearly an eight percent increase in the calf crop weaned when compared to the high level of supplemental winter feed (two and one-half pounds cottonseed meal plus three pounds of oats per head daily). The cost of producing 100 pounds of calf was nearly twice as much for the high level as compared to the low level (\$14.39 vs. \$7.62). Less disease loss and greater fertility were experienced among the cows fed limited amounts of supplemental feed. It required approximately  $l\frac{1}{2}$  years longer for the cows in the low level group to reach mature weights. Low level feeding also delayed calving although weaning weights were only slightly affected.

In this test half the heifers were bred to calve as two-year-olds and the rest as three-year-olds to determine the effect of age at first calving on lifetime performance. Thirty additional heifers (making a total of 120) were included in this phase of the experiment. In March, 1962, there were 23 of the two-year-old group and 22 of the three-year-old group still in the herd of the 60 starting in each group. The two-year-old group had weaned more calves (533 to 482) without much difference in weaning weight (476 to 485 pounds). (AH d2-12)

# 2. Management on Forest Range.

Cooperative work with the Forest Service and Georgia Coastal Plain Experiment Station on problems related to grazing beef cattle in the Southern pine forests is carried out at Alapaha, Georgia. Currently this work is divided into two phases, improving herd management on forest range and integrating livestock and timber production. Herd management studies compare burned and unburned native range supplemented with improved pasture.

Calves in herds whose dams were on burned range during the spring and summer averaged 407 and 440 pounds at weaning while those in herds which were on unburned range with limited improved pasture averaged 367 and 377 pounds at weaning or an average of 51 pounds more. There was very little difference in cow weights in the spring when they were placed on pasture. By late September when the calves were weaned the average weight of the cows on the burned areas had increased 150 pounds while those on the unburned range with limited improved pasture increased 115 pounds. There is a tendency for the cows on the unburned range to make up this difference during the fall and winter when they receive a fairly good winter ration.

Earlier attempts to graze pastures seeded among newly planted pine trees resulted in severe damage to the young pines. Trees were planted on 24 two-acre plots in 1957. These plots were clean cultivated until 1960 when they were seeded to pasture. Some difficulty was experienced in reestablishing the grasses in 1960 because of a long dry period which occurred in the spring soon after seeding. As a result there were some bare spots and contamination from common Bermuda grass. The pine seedlings grew well and in November, 1961, they averaged 16.5 feet tall and 5.6 inches in diameter at 6 inches above the ground. Grazing was started in April, 1961.

In 1961, the eight pastures having no pines produced 236 pounds of live weight gain per acre as compared to 156 pounds in the  $12 \times 12$  spacings and 208 pounds per acre in the  $20 \times 20$  tree spacing. There was no appreciable difference noted in the individual steer performance on the pastures having the three tree spacings. (AH d3-3, d3-4)

### 3. Productivity of Pastures Grazed by Cattle and Sheep.

Problems related to comparative productivity of pastures grazed by beef cattle alone, sheep alone, and by the two species in combination are being studied in cooperation with the Sheep and Fur Animal Research Branch and the Forage and Range Research Branch at Beltsville.

An Orchard grass-Ladino clover pasture was laid out in two replicates. There are four treatments and two stocking rates. The treatments are: cattle alone, sheep alone, cattle-sheep in a 1:5 ratio, and cattle-sheep

in a 1:1 ratio. The stocking rates are .75 and .50 acres per animal unit. The animals were grazed 163 days in 1961. Results indicate better animal gains on the lower stocking rate and with the steers and sheep grazing together.

In September, 1961, a trial was conducted to determine the intake and digestibility of the pasture. The following analyses are being made on the composite of forage and feces: % chromic oxide, % nitrogen, % lignin, units chromogen % organic matter, and % dry matter.

In 1962, the second year trial was to duplicate the first year's trial but because of prolonged drouth the animals had to be removed after 58 days to prevent permanent damage to the pastures. (AH b3-10)

### E. Management Practices

### 1. Interrupted Growth.

Carcass grades and meat quality of the animals which were retarded by feeding maintenance rations and then full fed were not appreciably affected by the 7-month period of low energy intake. However, there was an apparent decrease in tenderness as measured by shear and organoleptic tests. (AH d2-11)

### 2. Management of Cattle and Pastures for Beef Production.

Creep feeding experiments carried on for four years at Brooksville, Florida, with the Angus, Hereford, Brahman, and Santa Gertrudis breeds and a Brahman-Angus group showed wide breed differences. The creep-fed calves showed an advantage of 24 pounds over the noncreep-fed group. The average increase in weight of the creep-fed over the noncreep-fed was for Brahman-Angus 43 lb., for Hereford 37 lb., for Angus 17 lb., for Brahman 17 lb., and for Santa Gertrudis 15 lb. The carryover effect of creep feeding was studied using 69 head of 1959 heifers. The advantage of creep feeding was eliminated the following year after weaning. The noncreep-fed group was 6 pounds heavier at the end of the yearling year and made 36 pounds more gain per head than the creep-fed group. (AH d3-2)

### 3. Systems of Feeding and Management for Beef Production.

An experiment is being conducted at Beltsville in cooperation with the Dairy Research Branch to determine the relative merits of several systems of feeding and management for the production of beef from dairy and dual-purpose steers. Beef steers are also included in the experiments for comparative purposes.

Sixteen calves of four different breeds were put on two treatments from birth to six months. The six-month average daily gains for the calves

receiving maximum feed were as follows: 2.4, 1.8, 2.2, and 1.7 for Holstein, Angus, Milking Shorthorn, and Jerseys, respectively, and for calves on a limited-feed regime receiving milk replacer: 1.1, 0.8, 0.8 and 0.6 for the Holstein, Angus, Milking Shorthorn, and Jerseys, respectively.

At six months, calves in each group were divided into four groups as follows: (1) slaughtered at six months to obtain comparative information on body composition; (2) high concentrate ration; (3) roughage ration; (4) roughage until steers reach a given weight and then changed to the concentrate ration. These steers are all fed ad libitum. Incomplete data show that the average daily gains for the entire period were highest for the Holstein followed by Milking Shorthorn, Angus, and Jersey. Dressing percentages of these animals were rated in this order: Angus, Milking Shorthorn, Holstein, and Jersey. (AH d3-6) (Also reported in Areas 5 and 20).

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# SWINE - BREEDING Animal Husbandry Research Division, ARS

Problem: Improvements in the heredity of swine depend primarily on the intensity and accuracy of the selection that is practiced in choosing breeding animals and on the choice of a mating system that will maximize the rate of genetic improvement. Crossbreeding swine for the production of market animals has so proved its value that nearly 95% of the pigs marketed in the United States are currently some sort of crossbreds. Research with swine thus is faced with the dual challenge of devising breeding procedures that will increase further the advantages of heterosis or hybrid vigor generally shown by crossbred pigs, and also developing methods for increasing the effectiveness of selection within specific populations. It is essential, therefore, that experimental work on the effectiveness and practical usefulness of different breeding and selection procedures be continued to aid breeders in developing inherently more efficient strains of swine. Particular effort is needed on effective genetic means for producing pork with more lean meat and less fat without detrimentally affecting other production traits.

# USDA PROGRAM AND RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

The importance of swine breeding problems has resulted in the development of a closely coordinated research effort between the State experiment stations and USDA. Research is in progress at Beltsville, Maryland, cooperatively at Miles City, Montana, and the Regional Swine Breeding Laboratory with headquarters at Ames. Iowa. Regional Laboratory includes projects at ten States. Additional research in swine breeding is conducted at State experiment stations primarily in the North Central, Southern and Western regions. This is a continuing program of applied and basic research conducted by geneticists for the purpose of elucidating genetic principles and developing effective breeding techniques that will increase further the efficiency of swine with respect to productivity and carcass value. Investigations on selection and breeding systems include work on the economic importance of performance traits, their heritabilities and phenotypic and genetic correlations. The results of such studies provide the basis for emphasis given to different traits and their underlying factors in evaluating different kinds of selection and systems of breeding. Traits of major interest include sow productivity, pig viability, growth rate, feed efficiency, carcass composition and quality of meat.

Also a cooperative project with the National Research Institute of Animal Husbandry, Copenhagen, Denmark, provides for studies of fiber diameter of samples of muscle tissue from pigs in the selection experiment for high and low fatness at Beltsville.

A grant with the College of Agriculture, Poznan, Poland, provides for investigations on red blood cell and serum antigens to establish the mode of inheritance and relative frequencies of these antigens in certain breeds of swine. Its duration is for five years, 1962 - 1966, and involves PL-480 funds.

The Federal scientific effort in this area totals 9.5 professional man-years.

State experiment stations in 1961 reported a total of 23.6 professional man-years.

Industry and other organizations have shown considerable interest in the results from research on development of inbred lines and a registry association has been established for recording pedigree information on various inbred lines of swine. However, there has been practically no research by industry or other private organizations concerning the effects of different breeding systems or the relative value of different selection procedures. Not more than 2.0 professional man-years are provided by industry for swine breeding research.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

# A. Genetics and Interrelations of Performance Traits

# 1. Genetic and Phenotypic Parameters.

The inheritance of teat number and its relationship to maternal traits was examined in nine years' data from the Minn. #1 population at Grand Rapids. Heritability was .10 \( \frac{1}{2} \) .04 by dam-offspring regression and .23 \( \frac{1}{2} \) .20 by paternal half-sib correlation. Regressions of various maternal traits on total teat number were small and not significant for the traits considered. Significant regression values of .27 pigs weaned and 11.70 pounds of pig weaned on functional teat number were obtained when the effect of number of pigs born alive was removed. (AH al-17)

Heritability of litter size at birth was estimated in 1970 daughter-dam comparisons from three inbred lines of swine at the Minnesota station. The three estimates did not differ significantly at the .05 level from each other or from zero and the pooled estimate was .03  $\pm$  .07. In Wisconsin data heritability of litter size at 154 days of age was .20. (AH al-10 and al-17)

Estimates of heritability of daily gain from weaning to 140 days (or 154 days) were obtained from pigs on the Minn. #1, #2, and #3 breeds. No significant breed differences were found. A pooled estimate of .28 ± .06 (1419 df) and 0.14 ± .10 (451 df) was obtained from all breeds by gross and intra-sire regression of offspring on dam. (The former estimate is thought to be the better in these data.) Heritability was no higher in the Minn. #3 than from the other 2 breeds. The former is a recent derivative from a very broad genetic base. Other estimates of the heritability of post weaning growth rate were .40 (Wisconsin), .17 and .22 (Missouri), and .20 (Iowa). (AH al-4, al-10 and al-17)

At the Michigan Station, data from 80 individually fed boars were analyzed to study the relationship between daily feed consumption and feed efficiency, efficiency being defined as gain in live weight per unit amount of feed consumed. Boars with the larger appetites tended to be the less efficient (r = -.37). This was true in spite of the fact that the boars with the larger daily feed consumption were the faster gaining boars (r = .79) and the faster gaining boars were the more efficient (r = .29). Heritability of feed efficiency in Iowa data was .70, based on differences among litters of four pigs. (AH al-14)

Genetic correlations obtained at the Minnesota Station for post-weaning growth rate with feed efficiency, litter size at birth and average pig weight in a litter at weaning were -.22, .06 and .47, respectively. At Wisconsin the genetic correlation between number of pigs in a litter at 154 days and individual pig weight at 154 days was estimated to be .24. (AH al-17 and al-10)

Heritabilities estimated at the Oklahoma Station for carcass length, backfat thickness and loin lean area ranged from .4 to .6, while estimates obtained at the Iowa Station for dressing percentage, backfat thickness at the shoulder, middle of the back and over the loin ranged from .47 to .63. Phenotypic and genetic correlations between feed efficiency and growth rate were estimated at .50 and .71 at the Iowa Station. In contrast, negative correlations of -.4 and -.6 were obtained between these two variables at the Michigan Station. (AH al-4, al-8, al-10 and al-14)

At Missouri heritability estimates by the intra-sire regression of off-spring on dam were 1.01, -.17, -.10 and .15 for probed backfat thick-ness, weaning weight, conformation score and feed efficiency, respectively, and .83, -.06, .17 and .10 by midparent regressions. The realized heritability of probed backfat thickness from response to selection was .76. Estimates of genetic correlations were determined by the covariance between offspring and parents. Backfat thickness was genetically correlated with rate of gain by .70  $\pm$  .11, with feed efficiency by -.46  $\pm$  .23 and with conformation scores by -.93  $\pm$  .03. Large standard errors were found for the other genetic correlations among the traits studied. (AH al-6)

# 2. Genetic and Environmental Trends in Three Control Strains.

Genetic and environmental changes between generations and years and changes in maternal effects associated with age of dams have been measured in a breed-cross and a line-cross control strain at Beltsville and Hamprace control strain at Miles City, Montana, using the repeat mating technique described by Goodwin et al. (1955, 1960). Four litter traits (litter size, and litter weight at birth and at weaning), and two measures of individual growth rate (140-day weight and daily gain from weaning to 225 pounds), have been studied in the three strains. In addition, six carcass traits (backfat thickness, percent fat cuts, percent lean cuts, percent bacon, length of carcass, eye muscle area) were studied in the Beltsville strains. Results for the litter traits are based on 4 generations and those for individual pig traits on 3 generations. Changes in genetic merit between generations did not appear important except for post-weaning growth rate which declined rather markedly in the Hamprace strain. Matings within generations differed significantly for 140-day weight and daily gain in the line-cross strain and for litter size at birth, backfat thickness, percent fat cuts, percent lean cuts and length of carcass in the breed-cross strain. The linear component of environmental changes among years was significant for 140-day weight and daily gain in the Hamprace strain, for 140-day weight and percent bacon in the line-cross strain and for backfat thickness, percent fat cuts and percent lean cuts in the breed-cross strain. Maternal effects associated with age of dams were not apparent for any of the traits in the Hamprace strain, but two-year old sows in the Beltsville strains showed important advantages over one-year old sows in litter size and weight at birth and at weaning and in post-weaning growth rate. (AH al-11 and al-13)

# 3. Genotype - Environment Interactions.

Three trials including 240 pigs from 3 lines of breeding were conducted at the Oklahoma Station to investigate the importance of genotype - environment interactions in feedlot performance and various carcass traits. The two management treatments were a self-fed free-choice shelled corn and simple supplement ration on pasture and a self-fed pelleted vitamin--antibiotic fortified ration on concrete. No significant interactions were observed. Pigs raised on concrete, generally grew faster, had more backfat, larger loin eye, heavier belly, higher dressing percentage and a higher yield of primal cuts than the pigs fed on pasture. Pasture raised pigs, on the other hand, were sounder on their feet and legs. (AH al-8)

# 4. Size of Muscle Fiber as Related to Differences in Fatness.

Samples of the <u>longissimus dorsi</u> muscle obtained in 1960 and 1962 from representative pigs of the high- and low-fat lines in the

Duroc and Yorkshire breeds at Beltsville were used by the Forsogs-laboratoriet, Copenhagen, Denmark, to measure fiber diameter. In both breeds fiber diameter of samples in 1962 averaged higher than those in 1960, the respective averages being .073 and .068 mm. for Durocs and .081 and .076 mm. for Yorkshires. In both years high- and low-fat Yorkshire pigs exceeded high- and low-fat Duroc pigs, suggesting that a breed difference was present. Breed differences in both years suggest a genetic influence on fiber diameter. This primary work needs further study to clarify the picture regarding the relation of muscle fibers to carcass traits. (AH al-12)

Relationships between fiber size and muscle mass were studied at Nebraska. These studies indicated less promise for using muscle fiber size as a tool for selection for muscle mass than seemed likely from preliminary work. The need for accurate ways to estimate cell or nucleus number at an early age was a limiting factor. Biochemical work is needed in this area because of its importance to research in all species of meat animals. (AH al-7)

# 5. Pilot Experiments.

The 15th generation of a selection experiment at the Minnesota Station for 18-42 day growth in mice has been completed. Average weight gain in the selected line (S) has been 4 grams (40%). The observed improvement has agreed with a predicted value based on selection actually attained and an estimated heritability of 20%. improvement has been more rapid in later generations. Estimates of heritability were similarly higher in later generations. The increase in effective genetic variance is postulated to be the result of recombinations. The cross of the S-line with the long inbred A-line has not improved by half as much as the S-line (the expectation assuming only additive gene action). To ascertain if selection had worked on the maternal component, the S-line was reconstituted from the original inbred foundation stocks. The relative performance of the original and reconstituted stocks agrees with the estimate of genetic improvement that has been made. Reciprocal crosses of the two line stocks indicate that change in maternal influence has been small, if any. Hence, relative improvement for the S and S X A mice suggests a degree of nonadditivity of gene action. (Minnesota - AH al-17)

At the Michigan Station, a randomly-mated and an inbred strain of rats were formed from each of a two-strain and a three-strain cross. As inbreeding increased, the difference in weight gains between the randomly-mated and inbred lines increased with the randomly-mated line outgaining the inbred line from 4 to 34 grams in the three-strain population and from 12 to 21 grams in the two-strain population. For each 10% of inbreeding, a reduction in gain of 4.4 grams was found in the two-strain inbred line and a reduction of 2.5 grams in the three-strain inbred line. (AH al-14)

At the Indiana Station the possibility of using potassium-40 counts as means of estimating lean body mass was explored. In this preliminary work 98 pigs were observed at 5 ages, starting at 54 pounds live weight and terminating at 200 pounds when the pigs were slaughtered for carcass observations. The correlation between total K-40 count and live weight was .93, while simple correlations of K-40 count with the weight of ham, weight of the loin, and loin eye area were .51, .57 and .40, respectively. (AH al-3)

# B. Selection and Breeding Systems

# 1. Selection for Single Traits.

Selection for high and low fatness at Beltsville has been carried through 7 generations in two lines of Duroc pigs and through 5 generations in two lines of Yorkshire pigs. In addition, a randomly-selected control line is maintained in each breed by using 12 boars on 16 gilts as parents of each new generation.

In Durocs backfat thickness at a live weight of 175 pounds has increased from 1.49 inches in the foundation population to 2.01 inches or by 35% in the high-fat line and decreased about 18% to 1.22 inches in the lowfat line. Backfat thickness in seventh generation control line pigs averaged 1.50 inches. Realized heritabilities computed as the regression of generation means on respective cumulative selection differentials continued to suggest greater effectiveness of selection for high fatness than for low fatness, the two heritabilities being .72 and .46, respectively. When calculated in terms of deviations from the control, the heritabilities for the high- and low-fat lines continued to be more nearly equal, i.e., .63 and .58, respectively. Corresponding heritabilities for fifth generation high- and low-fat Yorkshire pigs were in both cases .36 when based on actual time trends, and .27 and .45 when the time trends were expressed as deviations from the control. These latter values are considerably lower than the corresponding ones of .53 and .72 for fifth generation high- and low-fat Duroc pigs. This suggests rather strongly that there is less genetic variation for fatness in Yorkshires than in Durocs.

Litter size and litter weight within the various lines differed little between 1961 and 1960. However, only 5 or 31% of 16 gilts bred in the Duroc high-fat line produced a litter in 1961, compared with 75% in each of the low-fat and control line Durocs, and 94, 75 and 100% in the three Yorkshire lines. Post mortem examination of three high-fat Duroc gilts revealed 9 partly decomposed embryos in one gilt and 11 and 14 in the other two. Any decline in reproductive fitness shown by this line as a result of its increasing fatness would thus seem to be more largely due to an increase in embryonic mortality than to a decline in ovulation or implantation rates.

Growth rate, as measured by daily gain from weaning to 175 pounds live weight, continued to be higher for low-fat than for high-fat Durocs (i.e., 1.44 vs. 1.38 pounds), whereas in Yorkshires high-fat pigs continued to excell low-fat pigs (i.e., 1.38 vs. 1.29 pounds).

Feed efficiency as measured by the amount of feed consumed per 100 pounds gain from weaning to 140 days of age continued to show substantial advantages for low-fat over high-fat pigs in both breeds. The respective averages for low-fat, high-fat and controls were 295, 328 and 304 pounds for Durocs and 295, 318 and 303 pounds for Yorkshires.

Carcass data continued to show selected lines diverging from each other and from the controls. Seventh generation high-fat, low-fat and control line Durocs averaged 2.06, 1.53 and 1.97 inches in backfat thickness; 28.5, 29.9 and 29.0 inches in length of carcass; 2.65, 3.94 and 3.68 square inches in loin eye muscle area; 37.0, 41.4 and 39.2% in yield of lean cuts; 17.2, 11.6 and 15.0% in yield of fat cuts; and 12.1, 10.6 and 11.2% in yield of bacon. Differences between 5th generation high- and low-fat Yorkshire pigs were in the same direction but smaller. High-, low- and control-line Yorkshire pigs averaged 1.86, 1.37 and 1.59 inches in backfat thickness; 30.7, 31.0 and 30.7 inches in length of carcass; 3.60, 4.06 and 4.01 square inches in loin eye muscle area; 40.2, 42.0 and 40.6% in yield of lean cuts; 14.3, 12.0 and 13.6% in yield of fat cuts; and 12.0, 10.8 and 11.2% in yield of bacon. (AH al-12)

At the Missouri Station, two generations of selection for low backfat thickness in two lines of Poland China swine have resulted in average decreases of 0.18 and 0.20 inches, respectively. Backfat thickness of second generation pigs adjusted to 175 pounds live body weight averaged 0.94 inches in one line and 0.89 in the other. (AH al-6)

At the South Dakota Station, selection for low backfat thickness in a Duroc line resulted in a decrease of .07 inches from 1.29 in 1960 to 1.22 in 1961. Backfat thickness adjusted to a live weight of 200 pounds has decreased by about .20 inches since selections were initiated in 1953. Carcass data suggest that there have been accompanying improvements in carcass length and loin eye area. (AH al-9)

The research in progress at the Iowa Station to study the response of swine to selection for a single trait in each of three purebred and three crossbred populations has shown relatively little differentiation and, as judged by comparison with the control lines, relatively little response to selection. The traits being selected for in the two sets of selected lines are increased litter size, increased growth rate and decreased backfat, with the control lines maintained by use of randomly-selected animals only. Further study of the selection differentials is in progress as the basis for possible revision of the design. High

level of disease is thought to be responsible for small selection differentials. (AH al-4)

# 2. Selection for Combining Ability.

Reciprocal recurrent selection programs at Beltsville, Maryland, and cooperatively with the Montana Agricultural Experiment Station at Miles City, Montana, are now in the fourth and fifth cycles of selection. The traits primarily considered in selecting animals for crossing are their dam's productivity, their own post-weaning growth rate and their own backfat thickness at a live weight of 175 pounds. Selection of animals for propagating each strain is based primarily on the performance of their cross progenies as measured by the size and weight of litters in which they were born and raised, their individual post-weaning growth rate, and in the case of the Beltsville project, also their carcass value as measured by yield of lean cuts.

Results obtained in the Beltsville project have shown some heterosis with respect to size and weight of litter and post-weaning growth rate. However, the advantages for crosses have been rather small and, except for litter weight at weaning, there is little evidence of an upward trend in the performance of crosses. Single cross gilts mated to control strain boars continued to show substantial advantages over controls in both litter size and litter weight and to a lesser extent in post-weaning growth rate. The advantages for litter size at weaning averaged 0.4 pig or 5.0% in 1957, 1.5 pigs or 20% in 1959 and 1.5 pigs or 19% in 1961. In post-weaning daily gain the advantages shown by pigs out of single cross dams averaged .07 pound or 5%; .05 pound or 4%, and .12 pound or 8%, respectively.

At Miles City, Montana, successive cycles of Hamprace x Yorkshire and Yorkshire x Hamprace crosses continued to show increasing advantages over contemporary controls in litter weight at weaning but showed little additional improvement in post-weaning growth rate. Starting with 1953 when the first reciprocal crosses were made, litter weight at weaning has increased at the rate of 8.0 and 7.6 pounds per year in Y x H and H x Y crosses, respectively, whereas in Hamprace controls it increased at a rate of about 3.6 pounds per year. There has also been some improvement in the productivity of Hamprace and Yorkshire sows selected to produce pigs required for the next cycle of crossing. The two groups of sows differed little in productivity when used for crossing as one-year olds, but Yorkshire "select" sows substantially excelled Hamprace "select" sows in each of the five years in which the two groups produced straight-bred pigs as two-year olds. The apparent lack of age of dam effects suggested by these results for the Hamprace "select" strain agrees with results of trials specifically designed to separate genetic from environmental changes in the Hamprace control strain, as indicated under A. 2 above. (AH al-11 and al-13)

A comparison was made at the Wisconsin Station between the productivity of sows from a reciprocal recurrent selection program and that of sows from a herd in which selection was for purebred performance only. In general, sows from the recurrent selection program exceeded sows from the other group but the results were not conclusive. (AH al-10)

In a revised reciprocal recurrent selection project at Oklahoma, one cycle ( $1\frac{1}{2}$  years) has been completed. Selection is for crossbred sow productivity as measured on crossbred half sisters of purebred females which propagate the two lines. Discarding animals for other things such as rhinitis, crooked legs and backfat thickness reduced selection differentials for sow productivity factors. If no culling had been done for other items, selection pressure for litter size would have been about .5 pig greater and litter weight at 21 days about 6 pounds more. The gilts saved actually showed small negative selection differentials, which without prior culling would have been positive by about .2 pig for litter size and 6 pounds for litter weight at 21 days. (AH al-8)

# 3. Development and Evaluation of Inbred Lines and Crosses.

At the Iowa Station, the Minnesota M line of Poland China swine with an inbreeding coefficient of 90% is being utilized to establish a new subline by Cesarian techniques for comparison with other inbred Poland China lines, blood antigen studies and genotype - environment interaction experiments. At the South Dakota Station, full brother sister matings within 11 sets of Yorkshire pigs resulted in the elimination of all but 3 of the stocks after 2 generations of such matings. In trials at the Michigan Station to test the effectiveness of intense inbreeding in evaluating potential sources of superior germ plasm, litters out of crossline gilts and sired by boars of a third line were compared with crossline litters out of inbred dams. The litters from crossline dams were heavier and larger at 154 days of age, but the carcass desirability was similar for the two kinds of pigs. Of the 5 inbred lines used in these trials, only 10 of the 20 sows and gilts bred to maintain the lines farrowed, compared with 12 of 15 crossline females that were bred to farrow in the same season. (AH al-4, al-9 and al-14)

At the Minnesota Station, progeny of 3-way cross boars (Minn. #3, Minn. #2 x Minn. #1) were compared with progeny of straight bred boars from these lines. Dams were comparable groups of Minn. #1's. The daily gain of 236 pigs sired by crossbred boars averaged 1.85 pounds, compared with 1.91 pounds for 221 pigs sired by inbred boars. Feed per 100 pounds gain was 372.8 and 371.2 pounds, respectively. Carcass data obtained on samples of pigs differed little between the two groups. The results thus show that the performance of pigs sired by crossbred boars is comparable to that of pigs sired by boars of the breeds from which the crossbred boars are constituted. (AH al-17)

The second year of an evaluation of the comparative performance of rotational crossbred pigs sired by boars of standard breeds with the Minnesota lines was completed. Crossbred pigs sired by Duroc boars were superior to pigs sired by Minn. #1 boars for growth rate, feed efficiency, and all measures of carcass performance. (AH al-17)

The following estimated contributions were made by various breeds to the Minn. #3 breed of swine: 31% Gloucester Old Spot, 20% Poland China C line, 14% Welsh, 12% Large White, 7% Beltsville #2, 6% Minn. #1, 5% Minn. #2, 5% San Pierre. Sixteen foundation animals contributed to breed formation. Estimated inbreeding reached 14% in the 5th generation. (AH al-17)

Data from 1256 litters were studied at the Oklahoma Station to determine the effects of line of breeding and sire on sow productivity as measured by number and weight of litter at birth and at weaning. Line averages differed significantly for some traits but no one line was superior to others in all seasons for any one trait. The results also showed no significant sire differences when sires were compared on the basis of their crossbred daughters' productivity records. (AH al-8)

Convergent improvement in a Hampshire line by addition of 2 to 4 outbred gilts representative of the breed has resulted in very slow changes in the line in performance and conformation traits. Top crosses exhibit hybrid vigor inversely as their relationship to the line increases. (AH al-9)

### 4. Environmental Influences as Related to Performance.

Inbred lines at the Iowa Station put through modified specific pathogen free (SPF) procedures showed increased productivity of dams. Respective averages for non-SPF in 1960 and SPF in 1962 were: 84.8 and 94.5 for percent bred, 69.1 and 87.6 for percent farrowed, and 76.8 and 85.2 for percent live pigs at 7 days. Crossbred SPF pigs transferred to a high disease level at 8 weeks showed almost equal growth rate with non-SPF pigs and required 15 pounds more feed/100 pounds gain than non-SPF pigs. The Eureka, South Dakota, field station has also been repopulated with SPF rotation-cross pigs. (AH al-4 and al-9)

At Nebraska, the performance of 172 crossbred SPF litters farrowed and raised in a central farrowing house was compared with 40 crossbred SPF litters farrowed and raised in individual houses. Pigs farrowed during the early half of any one farrowing period in the central farrowing house were significantly heavier at 56 and 140 days of age than those of the late half. No significant difference existed in the early and late half in individual houses. Pigs farrowed in individual

houses and fed to market weight according to age and size in groups of 50 grew more rapidly. Numbers of pigs alive at birth were low at all three Nebraska stations. A majority of gilts farrowed large numbers of mummified fetuses. Many complete resorptions occurred in late gestation. Despite the reproductive problems, the SPF herds at North Platte and Lincoln remained free of virus pneumonia and atrophic rhinitis. (AH al-7)

Selection differentials for rate of gain and backfat thickness were low in two Indiana inbred Chester White lines. Incidence of virus pneumonia was high. Disease in the Minn. #2 at Rosemount and Morris have importantly affected numbers of animals available for breeding in this line. A slightly less serious disease problem in the Minn. #3 also arose at Duluth. (AH al-3 and al-17)

Confinement and pasture management systems for swine were studied at Oklahoma. Results at 56 days indicated that death losses were fewer with confinement, but confinement gilts ate more feed and lost more weight than gilts on pasture. In post-weaning performance, pigs fed on concrete floors gained faster, required less feed per pound of gain, had more backfat, larger loin eye area, heavier bellies, higher dressing percentage, and a higher yield of primal cuts than pigs fed free choice corn and supplement ration on pasture. These differences were statistically significant. The lower cost of the pasture ration made the cost per 100 pounds gain considerably less on pasture than on confinement. Pigs on pasture were sounder on their legs and produced slightly leaner and longer carcasses than those raised in confinement. (AH al-8)

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# SWINE - PHYSIOLOGY Animal Husbandry Research Division, ARS

Problem. Continued improvement in efficiency of swine production is dependent on new information regarding the physiology of growth and reproduction as well as environmental adaptation. Particularly in the field of swine fertility considerable knowledge is needed regarding the development of artificial insemination, including semen and ova preservation and storage. Fertility problems in boars and sows seriously plague the efforts of the industry to produce pork at lowest cost. Development of new genetic aids for improvement of swine requires additional understanding of the physiological processes, particularly those involved in the growth and production of lean meat.

#### USDA PROGRAM

This is a continuing program conducted by physiologists on basic and applied studies on the physiology of reproduction, artificial insemination, effect of hormones on growth and development and the physiology of growth and development, particularly with respect to the mechanisms involved in fat deposition, muscular development and inborn metabolic defects. No work in this area is currently in progress at Beltsville due to a vacancy, but the program will be reactivated when qualified personnel can be obtained. Cooperative studies on the physiology of reproduction are included in projects of the Regional Swine Breeding Laboratory at Missouri and Nebraska, with informal preliminary investigations in others when opportunities for them arise.

The <u>Federal</u> scientific effort on research in this area totals 2.4 manyears. Of this number, 0.1 is on <u>physiology of reproduction</u>, 2.0 on <u>physiology of growth and development</u>, and 0.3 on <u>program leadership</u>.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State experiment stations in 1961 reported a total of 11.2 professional man-years divided among subareas as follows: physiology of reproduction, 4.6; environmental physiology, 2.4; other physiology, 4.2. This research is conducted by stations in four regions, with the North Central, Northeastern, Southern and Western Regions providing 5.7, 0.5, 4.2, and 0.8 man-years, respectively.

At the State stations, work in the subarea physiology of reproduction is based on the thesis that increased reproductive efficiency in swine is dependent primarily on an understanding of the nutritional and physiological influences on ovulation rate and embryonic survival. Fundamental studies are underway designed to yield information on endocrine events occurring at the time of ovulation, and physiology of

the uterine tract conducive to maximum embryonic survival. The effects of levels of nutrition at various stages of growth and development on ovulation rate and embryonic survival are also being studied.

Stress factors under study in the subarea environmental physiology include high- and low-ambient temperature, optimum temperature and humidity at different stages of development, and interrelationships of environmental temperature with nutrition and environment. The effects of cooling of males and females on conception rate and prolificacy is also being investigated. Additional studies in this area, including design of housing and equipment, are being conducted in cooperation with Agricultural Engineering. These are reported under Unit 1, Agricultural Engineering Research.

State stations are investigating the influence of inbreeding and cross-breeding on physiological mechanisms affecting growth and fertility. One station is determining the time and rate of development of differences in carcass characteristics in barrows and gilts, and the influence of feeding low levels of hormones on sex-influenced growth pattern. Extensive basic research is concerned with the nutrition and physiology of the developing swine fetus. Changes in total serum protein and serum protein electrophoretic patterns during fetal development are being investigated and histological changes of the gastrointestinal tract as they occur during development of the fetus are being characterized.

<u>Industrial</u> participation in swine physiology research is conducted by pharmaceutical manufacturers, mostly in the field of feed additives and the administration of hormones with particular emphasis on developing and testing new products. It is estimated that approximately 25 professional man-years are expended by industry in this work.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

# A. Physiology of Reproduction

### 1. Selective Fertilization and Mating Behavior.

Multiple sires were put with different groups of females at the Nebraska station to study the frequency of multiple matings and mating behavior. Multiple mating occurred in 65% of all litters. Order of mating had less effect on the relative number of progeny per sire than the apparent physiological phenomena of selective fertilization. Observations on mating behavior indicated more sex drive by Hampshire than Yorkshire or Minnesota #1 boars. Animals with more sex drive tended to rank higher in reproductive capacity. When boars that ranked high in social order in their group were paired with boars of similar rank, they accepted one another as equals. Fighting was most vicious among pairs of low-ranking boars. (AH al-7)

# 2. Factors Influencing Estrus and Fertility.

Gilts at the Missouri Station, implanted with 400 mg. progesterone and 200 mg. estradiol on the 7th day of pregnancy, showed no improvement in embryonic survival (31%) over controls (32%). another study, 22 sows were treated with Armour's Gonadotropin for control of estrus and ovulation. Of all recycled sows bred at the first estrus after treatment, nine were pregnant when slaughtered 25 days after breeding. Subcutaneous stilbestrol and progesterone estradiol implants failed to increase growth rate, but lowered backfat thickness. Previous investigations on the induction of superovulation in swine were continued with modifications of the type of hormone treatment employed. The injection of follicle stimulating hormone (FSH), pregnant mare serum (PMS), or human chorionic gonadotropin (HCG) at various stages of the estrus cycle failed to induce a significant increase in ovulation rate or litter size. The combined treatment PMS + HCG, gave an increase in litter size in a small sample of gilts. (AH al-6)

The effect of administering antibiotics at time of breeding on litter size was investigated in one experiment (29 animals). Alternate animals exhibiting estrus received a uterine flush of penicillin and dihydrostreptomycin. No improvement was noted in the treated animals. (AH al-6)

In one line at Nebraska, age at puberty ranged from 136 to 270 days. The correlation between age and weight at puberty was .82. Gilts fed a low energy ration at Missouri reached sexual maturity at a younger age and had more regular reproductive cycles than those on a high energy ration. Differences between the two groups in litter size at 25 days gestation were insignificant. (AH al-6 and al-7)

### B. Physiology of Growth and Development

# 1. <u>Hormone Treatments</u>.

Poland China barrows were used at the Missouri Station to study the influence of subcutaneous stilbestrol and progesterone - estradiol implants on growth rate and carcass composition. Stilbestrol and high level progesterone - estradiol tended to reduce growth but the effect was small. Progesterone - estradiol decreased backfat thickness and tended to increase leanness. In another study a method was developed for measuring thyroxine secretion rate of swine. The average daily thyroxine secretion rate for growing finished pigs was found to be 0.46 mg. per 100 pounds body weight. (AH al-6)

# 2. Serum Cholesterols.

Total serum cholesterols were measured at Indiana in two groups of Chester White swine between 92 and 182 days of age during two seasons. No differences were observed between castrated males and intact prepubertal females. Means and variance were greater in the winter months than in the summer and early fall. Litter differences were indicated. Cholesterol values for all litters changed with increasing age in a similar (+ or -) manner. The direction of change was determined by season. The role of serum cholesterol in lipid metabolism and its relation to weight, gain, feed efficiency and carcass traits was studied. In general, the relationships to production and carcass traits were too low to be very useful for predicting performance by individuals. (AH al-3)

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# SWINE - NUTRITION AND MANAGEMENT Animal Husbandry Research Division, ARS

Problem. The changing demands of the consumer to pork with a high proportion of lean is requiring major changes in the nutrition and management of swine. Furthermore, the use of materials other than lard has greatly reduced the demand for fat-type hogs. Along with the change in genetic makeup which must be made, basic facts concerning metabolic functions require investigation, and the basic nutritional factors which influence growth and carcass composition need to be identified and evaluated. These require information on quantitative and qualitative requirements at various growth stages and the changes in requirements to adjust for altered levels of other nutrients or modified environment. To meet the competition of other foods, including other meats, the nutrition and management of swine must constantly be aimed at improvement of feed and labor efficiency.

#### USDA PROGRAM

This is a continuing program conducted by biochemists, nutritionists and animal husbandmen, investigating basic and applied problems in swine production related to nutrition, metabolism and management. Work is in progress at Beltsville, Maryland, and cooperatively with the Agricultural Engineering Division, the Michigan Agricultural Experiment Station, the National Institutes of Health and the Food and Drug Administration. These studies contribute to the establishment of nutrient and mineral requirements and the relation of different components of the diet to each other; to the development of more efficient and economical rations; to the relation of genetic differences to dietary requirements; and to the role swine may have as an experimental animal for the investigation of health and dietary problems in man.

The total <u>Federal</u> scientific effort in this area amounts to 7.9 professional man-years. Of this number, 0.1 are devoted to <u>digestion</u> and <u>metabolism</u>, 1.5 to <u>concentrates</u>, evaluation and <u>utilization</u>, 1.5 to <u>forage evaluation and utilization</u>, 3.0 to <u>nutritional requirements</u>, 1.0 to <u>management practices</u>, equipment and facilities, and 0.8 to <u>program leadership</u>.

A research contract with Michigan State University, East Lansing, Michigan, provides for evaluation of certain feedstuffs as sources of fiber in modifying carcass quality factors and on efficiency and economy of swine gains.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

<u>State experiment stations</u> in 1961 reported a total of 41.0 professional man-years divided among subheadings as follows: digestion and metabolism

2.9, concentrates 4.8, forages 3.2, nutrients 21.9, and management practices, equipment and facilities 8.2. This research is underway in all four regions, but the major effort is in the North Central (20.2 professional man-years) and Southern (15.4 professional man-years) regions.

Digestion and metabolism studies deal with the physiology of the digestive system, how it functions and factors which alter its function, and the basic biochemical reactions by which nutrients are metabolized. The efficient use of concentrates, feed grains in particular, is of primary concern because of the high proportion of concentrates in swine rations. The ways in which specific feeds and byproducts may be used, together with mineral, vitamin and amino acid supplementation required in the diet, constitute main lines of research.

Investigations on forages include ways to utilize them, including evaluation of specific forages as swine feeds, to provide cheaper vitamin and mineral supplementation and reduce energy intake with labor-saving self feeding. Self-feeding of diets high in energy leads to obesity and poor performance in brood sows and also brings many hogs to market that are too fat. This research has the purpose of utilizing fibrous feeds for more efficient production of swine in the production of high-quality pork.

The requirements of swine for specific minerals, vitamins, amino acids and energy (carbohydrates and fats) together with the interactions and toxicities which occur among them, constitute the main lines of swine nutrition research. Other areas of major work are: The use of feed additives (hormones, antibiotics, enzymes, etc.) in improving growth and feed efficiency; the effect of nutritional factors upon various stages of the life cycle (reproduction, gestation-lactation, etc.) and upon disease resistance and/or antibody formation.

The ways of managing the swine enterprise for more efficient operation are being investigated. The advantages to be gained by "early weaning" of pigs, ways in which farrowing times may be varied for marketing at more desirable times and for more efficient use of feeds such as pasture and combinations of pasture and drylot feeding, management of the sow in gestation and lactation, and parasite control through proper management are all under study.

<u>Industrial</u> participation in swine nutrition and management research is by feed manufacturing and pharmaceutical companies and is mostly in the field of feed additives and ration comparisons with particular emphasis on developing and testing new products. Some work is conducted on nutritive requirements, metabolic disorders and endocrine functions. This research activity, exclusive of quality control and ration calculation efforts, is estimated at 75 professional man-years.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

## A. Digestion and Metabolism

# 1. Source of Fiber (Michigan and Beltsville)

The contract at Michigan, on relationship of selected dietary fibers to rate and efficiency of swine gains and to production of lean pork carcasses, has been completed. The corn and cob meal diet gave the best overall performance producing intermediate rate of gain with carcass quality and feed efficiency at or near the top rating for the experiment. The diets which proved inferior included the corn meal (control) and other diets which used oats or wheat bran or alfalfa meal as principal sources of fiber. The results of this research have been submitted to the Journal of Animal Science for publication. (AH a3-17 (C))

The study at Beltsville concerning use of harvested forage and fiber as management tools suggests that corn and cob meal may have practical applications for rearing breeding stock but is not as well adapted as alfalfa for gestation diets. (AH a2-4)

## 2. <u>Digestion Trials</u>.

In past years at Beltsville, investigations of metabolic processes and digestive capacities of swine have been limited by inadequate collection equipment. A metabolism cage has been developed which can be adjusted for separate collection of urine and feces from male pigs ranging in size from weanlings (56 days) to market hogs of approximately 200 to 225 pounds. The cage, harness and procedures evolved over a period of four years and are perfected to a degree that contamination or losses of excreta occur only about 2% of the time. The use of stainless steel feed and water pans plus floor screens and polyethylene collection equipment make these facilities equally valuable for trace mineral metabolism studies, balance studies, energy work or any research utilizing total collection of excreta. This development has increased the research scope at this station and expansion to its full potential can contribute to the total research effort in the field of digestion and metabolism of swine at other laboratories as well.

# B. Concentrates - Evaluation and Utilization

## Cottonseed Meal.

In the investigation at Beltsville, Maryland, directed at improvement in safety and use of cottonseed meal as a swine feed, enlargement of the heart has been such a consistent finding in autopsies of swine suffering from cottonseed meal toxicity that weight of heart in proportion to body weight has been accepted as the most reliable diagnostic indicator of gossypol toxicity. Tissue analysis shows the heart enlargement to be due largely to increased water content and limited observations of sodium and potassium content of blood serum and heart tissue support the hypothesis that this edema is due to changes in cell permeability. Liver enlargement which is a frequent though less consistent toxic symptom is due to increases in both water and dry matter content.

The pigment, gossypol, in cottonseed has long been recognized as toxic to monogastric animals. However, many experiments have failed to show consistent correlation between toxicity of certain commercial cottonseed meals and their chemically determined gossypol content. Since gossypol is readily oxidized it seems logical to assume that antioxidants might affect physiological action of gossypol. Several rat feeding tests were conducted to measure the effect on gossypol tolerance of flavonol glycosides - antioxidants naturally present in cottonseed. Statistically significant variation in weight changes of experimental rats supported the conclusion that the flavonol glycosides, rutin and iso-quercetin, recently identified in cottonseed, might affect the level of gossypol which could be tolerated in diets of monogastric animals.

Further studies in collaboration with workers of S. R. R. L. at New Orleans showed that: cottonseed containing raffinose was reduced in lysine content and nutritive quality of proteins when heat was applied; 1% gossypol was less effective than 10% raffinose in destruction of lysine in cottonseed meal, but these concentrations were comparable in reducing level of free epsilon-aminolysine in cottonseed proteins; and finally the nutritive index of cottonseed meals (by the rat repletion method)showed poor correlation with total lysine but high correlation with free epsilonamino groups of lysine of the protein. (AH a3-16)

#### C. Forage Evaluation and Utilization

## 1. Forages in the Diet of Breeding Animals.

This experimental series at Beltsville concerns use of forage and fiber as management tools. An optimum method for managing female swine through growth and gestation should combine economy and high level of reproductive performance. Properly supplemented pasture or silage fulfill both requirements reasonably well and this experiment is designed to achieve similar results with a relatively high level of ground alfalfa hay (55 to 60%) to provide approximately 17 to 18% crude fiber in the diet. This alfalfa diet, fed ad lib. as a pellet, is compared with a meal diet based on ground ear corn, also fed ad lib., and a conventional corn diet which is hand-fed at a restricted level. In this study which involves management and nutrition the high fiber alfalfa diet (15 to 17%) was rather drastic for young swine and both growth and sexual maturity were retarded. However, these gilts had

nearly caught up in weight to those reared on the conventional diet at first farrowing and to date reproductive performance is nearly identical. Gilts fed ad lib. on the corn and cob meal diet (about 8% fiber) tend to grow at a somewhat slower rate than those on the conventional diet (about 4% fiber) but after being bred they gain more than is considered desirable. This seems to be borne out by a slightly but consistently inferior reproductive performance averaging about  $\frac{1}{2}$  pig less per litter at 56 days of age. A final evaluation of these diets will require some additional compilation and analysis to determine whether lower costs per pound and savings in labor costs compensate for the additional pounds of total diet consumed by ad lib. feeding of medium and/or high fiber diets. (AH a2-4 and a3-17 (C) )

# D. Nutritional Requirements

## 1. Trace Mineral Requirements and Interrelationships.

The investigations at Beltsville have included studies on (a) the utilization and retention of zinc in pigs as influenced by the level of calcium in the diet and by dietary supplements of phytic acid and of ethylenediamine tetra acetic acid; and on (b) the interrelations between the amount of zinc stored in the tissues, the phosphatase enzymes in blood and tissues and purine metabolism as measured by the urinary excretion of end products.

Tentative conclusions drawn from the results of these studies are as follows: (1) On diets in which only zinc and calcium were varied, at zinc levels of both 30 p.p.m. and 80 p.p.m., less zinc is retained at 1.0% calcium than at 0.5% calcium as indicated both by zinc balance studies and by measurement of zinc stored in the liver. (2) On these same diets the alkaline phosphatase activity of the blood serum is correlated with the zinc in the liver. (3) The phosphatase activity of kidney and of gut in general follows the same trend as the alkaline phosphatase in the serum but appears to be less consistent in relationship to the zinc in the liver. (4) The phytase activity of the gut seems not to be influenced either by the level of zinc in the diet or by the amount of zinc stored in the liver. (5) The addition of ethylenediamine tetra acetic acid to a high calcium - low zinc diet has the same effect on the phosphatase enzymes as the addition of zinc, in some litters studied whereas in other, it appears to be without effect. (6) The addition of phytic acid to the diet appears to precipitate a zinc deficiency as indicated by lowered phosphatase activity in blood and tissues in some litters but is without effect in others. (7) The excretion of uric acid, both in total amount per unit of body weight and in proportion to the amount of allantoin, appears to be related to the level of zinc in the diet, that is, it is greater where there is some evidence of zinc deficiency. (AH a3-12)

## 2. Pantothenic Acid Requirements.

The final compilation and analysis of results from this study at Beltsville confirm the tentative recommendation, stated in last year's report, of 5.4 mg. of pantothenic acid per pound of complete diet for female swine during gestation. This is only slightly below the figure (6.0 mg.) given in the 1959 revision of N. R. C. Pub. 648 on Nutrient Requirements. Gilts which were reared on diets containing 4.5 mg. of pantothenic acid (P.A.) per pound of diet rarely showed deficiency symptoms but almost one-third of them failed to produce pigs. Survival rate of pigs from these gilts was fairly high but a large percentage of these sucklings had developed incoordination and irregular gaits by weaning time at 56 days of age. Since gilts receiving 5.4 mg. of P.A. per pound of diet had a higher survival rate of offspring which did not show deficiency symptoms it is apparent that the 4.5 mg. level of P.A. is borderline or slightly inadequate to support optimum reproductive performance and that a 5.4 mg. level is adequate and significantly superior to 4.5. Experimentation is completed and a report is being submitted for publication in the Journal of Animal Science. (AH a3-11)

# 3. <u>Nutritional Status of Baby Pigs and Susceptibility to Infection</u>.

Michigan researchers have been partially successful in rearing germ-free pigs. The original procedure, employing hysterectomy with the gravid uterus passed through an antiseptic lock into the sterile environment, has been largely replaced by Cesarean sections performed through the bottom of a plastic isolator cemented to the abdominal wall of anesthetized sows. The diets fed newborn pigs consisted of cows' milk fortified with vitamins and minerals, with or without added casein and butter, which were autoclaved before feeding. To date two pigs have been reared to seven weeks in a bacteria-free environment and 16 others to three weeks of age. A number of additional pigs have been reared in the presence of one or two species of bacteria.

Pigs raised under farm conditions were significantly heavier than the artificially reared pigs, but though minor differences were found in weight of various organs expressed in terms of body weight, relative organ weights were quite similar regardless of method of rearing or bacterial association in the environment. This project closed effective June 30, 1962, with the termination of Regional Project NC-13. (AH a3-14)

As reported last year, a similar project at Illinois was rendered inactive by personnel changes, transfers and completion of graduate projects. The decision to terminate NC-13 made it inadvisable to resume work and the project was officially discontinued in August, 1961. (AH a3-15)

## E. Management Practices, Equipment and Facilities.

# 1. Portable Air Conditioner to Relieve Heat Stress at Parturition.

Cooperative studies with the Agricultural Engineering Division designed to appraise effects of temperature stress on sows at parturition and to evaluate methods of alleviating this stress have been included as adjuncts to nutrition experiments at Beltsville. The use of an air-conditioned maternity room  $(70^{\circ} \text{ F.} \pm 5^{\circ})$  from the 109th day of gestation until pigs reached 3 days of age eliminated symptoms of acute heat stress and permitted reproductive performance in hot weather approximately equal to that obtained during cool periods of early spring or late fall.

Use of an ordinary one-room air conditioner, mounted on a wheeled frame and with flexible ducts for discharging cooler air at one or more farrowing stalls, was reported last year. The reduction of heat stress from onset of labor through parturition was estimated through observation of behavior but was not supported by any quantitative measurements. On June 8, 1961, three sows were occupying adjoining farrowing stalls with air temperature at 84° F. and 50% relative humidity. Sow #155 was showing restlessness indicative of onset of labor pains with a rectal temperature of 101.30 F. and respiration rate of 120 to 160. The use of 2 cold air ducts reduced respiration to 108 in 15 minutes, but the rate increased slightly in the next hour to about 130 then fell again to average about 100. This sow had a litter of 15 pigs born alive during the night. Sows #178 and #9887 showed no restlessness and use of 1 duct each maintained their respiration rate between 10 to 16 and 60 to 80, respectively, for one hour. At this time the duct assigned to #178 was removed and added to #9887. This change caused respiration rate for #178 to rise from 12 to 100 while #9887 fell from 60 to 28. These limited data illustrate the physiological changes obtainable from the modification of environmental factors such as air temperature and humidity. (AH a2-3)

# 2. Ventilation of Buildings for Summer Feeding.

Previous to installation of a ventilation system in building #208, seasonal differences were observed between summer feeding trials and winter feeding trials. Although genetic differences and dietary variables preclude any direct comparisons it seems noteworthy that daily gains from weaning to  $225 \pm 10$  lb. average between 1.40 and 1.55 lb. for summer trials previous to ventilation and approximately 1.60 to 1.75 lb. for winter trials and the two summer trials completed since ventilation was installed. These averages apply to our better diets with good quality experimental animals. (AH a2-3)

### PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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- Stevenson, J. W., Davey, R. J., and Hiner, R. L. 1960. Some effects of dietary levels of protein and alfalfa meal and of antibiotic supplementation on growth, feed efficiency and carcass characteristics in swine. J. Anim., Sci., 19, pp. 887-897.
- Hoefer, J. A., Hines, R. H., Pearson, A. M., and Luecke, R. W. 1960. The effect of full feeding to 125 pounds followed by restricted feeding to market weight. 5th Ann. Mich. Swine Day Report AH 56.

## Concentrates - Evaluation and Utilization

- Cabell, C. A., and Earle, I. P. 1960. Amino acid supplements and gossypol tolerance in the rat. N. Atlantic Section Proceedings. Vol. II, ASAP.
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- Martinez, W. H., Frampton, V. L., and Cabell, C. A. 1961. Effect of gossypol and raffinose on lysine content and nutritive quality of proteins in meals from glandless cottonseed. Agric. and Food Chemistry., 9, pp. 1, 64. Jan./Feb.

### Forage Evaluation and Utilization

None

## Nutritional Requirements

- Waxler, G. L. 1961. Research on rearing specific-pathogen-free and germ-free swine. Proceedings Conference on Application of Caesarean-Derived Animals to Disease Control in Livestock and Laboratory Animal Production.
- Waxler, G. L. 1961. Studies on gnotobiotic pigs. I. A technique for rearing gnotobiotic pigs. II. A comparison of body weights, organ weights, and some histological features of gnotobiotic and farm-raised pigs. Ph. D. Thesis. Michigan State University.

## Management Practices, Equipment and Facilities

None

# PRODUCTION INFLUENCES ON ANIMAL PRODUCTS Animal Husbandry Research Division, ARS

Pork, beef and lamb meat are excellent protein foods and most American diets are built around them. However, these meats are each criticized by the consumer for too much fat covering, lack of a bright red color, tenderness and flavor. The choice cuts and kind of meat are directly reflected in the demand and in the price of the product. Similarly, milk, eggs, poultry meat, wool and fur are demanded by the public in a high relationship to the desirability of their traits whether they be nutritive or functional. Many of the production practices directly affect the characteristics of animal products. Breed differences in butterfat and color of milk, nutritional effects on color of egg yolk, environmental stress on strength of wool, castration effects on flavor, color and tenderness of meat are well known production influences on animal products. Many other effects of production practices, however, are not so well understood but may be of considerable economic importance. Effective measures of evaluating quality and quantity differences are very important parts of this effort.

#### USDA PROGRAM

This is a continuing program conducted by food product technologists, wool and fiber technologists, biochemists, chemists, physiologists, statisticians, and animal husbandmen engaged in both basic and applied research designed to develop methods and information which will be useful in evaluating quality and quantity of animal products and will aid in livestock production. Research on beef, veal, lamb and pork is directed at the influence of selection and breeding, nutrition, physiology, management, and other production variables on carcass and meat quality. Standards are being applied and adapted for appraisal of slaughter animals, of carcasses, and of meat cuts. The objective of the work with poultry and eggs is to ascertain those factors of nutrition, breeding, and management which contribute to the initial quality of poultry products and their capacity to retain that quality. Studies with wool, fur, and fiber are conducted to determine the physical, chemical, and biological structures and properties of wool and other animal fibers as influenced by production factors. on humane slaughter was initiated to develop information and techniques on preslaughter handling, restraining, immobilizing, and dispatching of hogs, cattle, and sheep, in order to determine the most effective procedures for meeting the requirements of the humane slaughtering law and the influence of the effect of these procedures on the quality of the meat. The work is conducted at Beltsville, Maryland; Dubois, Idaho; Fort Wingate, New Mexico; and in cooperation with eight State experiment stations. Cooperation is also carried out with the Eastern and Western Utilization Research and Development Divisions, the

Human Nutrition Research Division, and the Market Quality Research Division.

The <u>Federal</u> scientific effort devoted to research in this area totals 16.4 professional man-years. Of this number 5.4 are devoted to <u>beef</u>, 1.3 to <u>lamb</u>, <u>mutton</u>, and <u>chevon</u>, 4.0 to <u>pork</u>, 1.0 to <u>poultry</u> and <u>eggs</u>, 1.7 to <u>wool</u>, <u>fur</u>, and <u>fiber</u>, 1.6 to <u>humane slaughter</u>, and 1.4 to <u>program leadership</u>.

Contract studies were completed during the year with the State Experiment Stations of Nebraska and South Dakota. The work in Nebraska was initiated to study relationships of certain live animal and carcass characteristics. At South Dakota the studies estimated the genetic and phenotypic relationships of carcass characteristics, growth, and conformation traits. Each of these studies constituted funds equivalent to .1 professional man-years.

A contract is in progress with the Wyoming State Experiment Station regarding the evaluation of lamb carcasses. Funds supporting this contract amount to .2 professional man-years.

A grant with the Polish Academy of Sciences in Poland provides for studies on the color of pork as influenced by heredity, sex, age, feeding, and management. Its duration is for five years (1960-1964) and involves PL 480 funds with \$42,784 equivalent in Polish zlotys.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State experiment stations reported a total of 17.9 professional manyears of which 7.7 are on studies relating to beef, 3.1 lamb, mutton, and chevon, 2.8 pork, and 4.3 poultry and eggs. Comparisons are being made of grass fattening, drylot fattening, or combinations of these, as influences on carcasses and meat characteristics. Studies are being conducted on varying the length of heavy silage feeding preceding finishing with a high energy ration, creep feeding versus no creep feeding during the nursing period, and various combinations of ration ingredients with and without adjuvants. Research is in progress on the influence of ratios of protein to energy and total feed consumption on carcass characteristics of swine. Still other studies are on the evaluation of various criteria of selection for superior meat type swine. Research on egg quality includes work on the causes and prevention of blood and meat spots and undesirable yolk coloration. The effects of different egg washing techniques on interior quality are also being evaluated. Studies have been undertaken on the effect of nutrition and management on chicken and turkey carcass traits, such as skin pigmentation and a desirable amount of fat. A number of breeding projects contributing to regional research projects are designed to determine the effectiveness of selection and improving carcass

traits and the effect which selection of one carcass trait has on other carcass traits. Several stations are studying the pattern of growth in different breeds and crosses of sheep as affected by feed, sex, castration and type of birth.

The activity by <u>industry</u> in the field of animal products is generally in the field of product processing and marketing. There are a few studies regarding the influence of production practices on product characteristics. One of the larger packing companies is carrying out an extensive beef improvement program including evaluation of carcasses for production of muscling, absence of waste, desirable ratio of fat to lean, and tenderness. Also, the packing industry cooperates extensively with publicly supported experiment stations in the grading and evaluation of carcasses resulting from various nutrition and management studies. Several textile mills conduct work on wool traits in sheep, including clean yield, fiber diameter and fiber strength. The number of professional man-years involved in industry effort on this area is estimated as 4.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

## A. Beef

# 1. Tenderness.

This research is being conducted at Beltsville and in cooperation with the Agricultural Experiment Station of Texas A. and M. Work was continued on variations in content of individual free amino acids in muscle tissue and their association with tenderness. A statistical study has been made on data from 209 animals. Considerable variation was found in animals from different sources. No correlation was found between the relative amounts of leucine-isoleucine and panel tenderness score on beef samples from dual-purpose steers averaging approximately 22 months of age in tests at Lewisburg, Pennsylvania. Samples obtained from the breeding experiments at Front Royal, Virginia, and Miles City, Montana, showed correlation coefficients of .35 and .36, respectively. Beef from Beltsville experiments in nutrition showed a correlation coefficient of .56 in relation to panel tenderness score. These results indicate that the relationship between the relative amounts of certain free amino acids in meat serum and tenderness is not as simple as one might hope. (AH d4-6)

A recently developed instrument modification for testing tenderness of meat samples referred to as Slice Tenderness Evaluator (STE) has been used to test meat slices of roasts from 74 beef animals. It was noted that higher correlations with panel tenderness score were obtained when the meat was tested parallel to the muscle fiber orientation than when tested perpendicular to the muscle fiber (with the

grain rather than across the grain). Although the shearing action of the device was related to subjective tenderness evaluations, the puncturing values added significantly to the overall value of the measurement. Preliminary observations suggest the existence of tenderness differences among the dorsal, medial and lateral locations of the loin-eye muscle of the 9-10-11 rib cut.

It has been suggested in a study of 27 beef roasts that the internal meat temperature at which the roast is served to the panel has little or no effect upon the panel members to render similar findings as regards palatability characteristics. The panel was equally effective in scoring meat samples served either warm or chilled. (AH d4-4)

The study of collagen content and its relation to tenderness was continued at Texas A. and M. The results showed that the Longissimus dorsi muscle contained less collagen nitrogen than the Biceps femoris muscle in the raw steaks and in steaks cooked to final temperatures of 61° and 80° C. The rates of conversion of collagen to gelatin were similar in both muscles. There were large variations among animals in collagen nitrogen content and in the percentage transformed to gelatin during cooking. The trend of panel scores and collagen content were toward increased tenderness with increasing temperature for each of the two muscles. (AH d4-5)

## 2. Composition.

Measurements for predicting actual fat thickness and loin-eye area have been summarized on approximately 256 cattle. Ultrasonic observations of lean depth made at two, three, four, and five inches off the midline over the 12th rib of the cattle, correlated significantly (P < .01) with the traced loin-eye area. Simple correlation coefficients were .30, .30, .29, and .31, respectively. Higher simple correlations between ultrasonic fat measurements and actual fat thicknesses on these cattle were .46, .61, .60, and .55 for the two, three, four, and five-inch locations, respectively. At a point three inches off the midline, the ultrasonic fat and lean measurements were both significantly related to actual fat thickness with a multiple correlation coefficient of .74. Multiple correlations at other locations were slightly lower. The only ultrasonic measurement location of significant importance in the regression of ultrasonic measurement on loin-eye area was at a point four inches off the midline. The multiple correlation coefficient for this relationship was .80.

The chemical determination of creatinine content of the blood of slaughter animals is being studied as a possible method for determining live animal composition. An analysis of the data from 180 cattle obtained over a five-year period, shows that rations and years accounted for 42.7 and 32.0% of the total variation in creatinine content. Sex was a relatively unimportant source of variation. (BS 3-34)

# Supplementary Information on

## PRODUCTION INFLUENCES ON ANIMAL PRODUCTS

# A. Beef

- 3. Quality and Quantity of Meat as Affected by Production
- a. Beef from Beef, Dual-Purpose and Dairy-Type Steers

Preliminary carcass muscling and palatability results on 40 steers, in a study comparing the production of beef from beef, dual-purpose and dairy-type steers, indicate that the beef (Angus) steers possessed larger loin eyes per hundred weight of carcass than dual-purpose, (Milking Shorthorn) and dairy- (Holstein and Jersey) type steers. Angus and Jersey steers usually had higher marbling scores than Holstein and dual-purpose Shorthorn steers. No significant breed differences were noted for meat flavor, aroma or juiciness. Jersey and Angus steers were more tender than Holstein or dual-purpose Shorthorn animals as judged by the taste panel. Meat from the Angus steers studied was only slightly more desirable in overall palatability than meat from the other three breeds.

Some interesting differences in composition between these four breeds was found in the 18 check animals slaughtered when they were approximately six months of age. The steers which received milk the first six months had carcass weights nearly three times as heavy as those on a milk replacer. The percentage of separable fat in the carcass reflected the same difference, except for the Holsteins, which had less than twice the fat than those receiving milk. The ether extract fat of the lean tissue, an important factor in juiciness, and flavor of lean was most striking. For example, E.E.F. of the eye muscle in Angus calves was 4.30 and 0.91 percent for milk fed and milk replacer. For dual-purpose Shorthorns the values were 5.55 and 0.73 percent. However, in case of Holstein steers the differences were less, 5.46 and 2.13. Similar differences were found for E.E.F. in the lean of the round and remaining carcass lean. There were no Jersey steers on milk replacer. These results are based on only two or three animals from each breed and from the two rations. (Also reported in Areas "Beef Cattle - Nutrition and Management" and "Dairy Cattle - Breeding." (AH d3-6)

# b. The Composition and Tenderness of Meat from Steers when Fattened on a Ration Supplemented with Stilbestrol.

A study of the chemical composition of the meat and tenderness of the standard 9-10-11 rib sample from 12 pairs of identical twin steers was completed. One twin of each pair was fed a standard fattening ration. The other twin of each pair was fed a ration supplemented with either stilbestrol, stilbestrol-aureomycin or stilbestrol-terramycin. The chemical analysis consisted of moisture, protein, ash and ether extract fat. A statistical analysis of the data indicates there was no appreciable effect on meat composition or tenderness due to supplementing a fattening ration with stilbestrol. The paired 9-10-11 ribs were used as samples. (AH d4-7)

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stilbestrol. The paired 9-10-11 ribs were used as samples. (AH d4-7)

## c. Effects of Sex.

A study of certain meat quality factors in beef samples from 10 pairs of identical twin bull calves, consisting of 10 bulls and 10 steers all slaughtered at approximately the same weight, was made. A general statement from this analysis is that steers had smaller rib eye areas, less weight of preferred cuts, less total lean and more fat than bulls. Steers had more tender meat as determined objectively by the Warner-Bratzler shear and subjectively by a panel of trained judges, had more juicy meat that was richer and the meat was brighter colored. (AH d4-7)

### d. Effect of Ration.

A summary was made on data collected over a two-year period on 54 steers fed corn supplemented rations. Steers fed corn plus corncob meal had distinctly larger average pancreas than those fed shell corn plus Vitamin A. Slaughter weight was inversely related to pancreas weight in these rations. Steers fed shelled corn plus 10% soybean meal had slightly greater primal yields than steers on the other two rations. Steers on corn plus corncob meal ration had distinctly larger eye muscle areas both years. Steers fed shelled corn plus Vitamin A had slightly greater percentage of separable lean both years. (AH d4-7)

# B. Pork

## 1. Tenderness.

In studies of quality in pork, electrical resistance of muscle tissue is being investigated. Palatability determinations of selected heated pork loins used in this study revealed that rather wide variations were present. Objective measurement of tenderness by the Warner-Bratzler shear and the tenderness press methods on 83 hogs from the reciprocal recurrent selection experiment showed a significant decline in tenderness as the percentage of Landrace blood increased. Panel evaluation of tenderness and juiciness showed significant interactions between breeding groups and sex.

The Slice Tenderness Evaluator (STE), being developed at the Agricultural Research Center in cooperation with A.M.S., was used to determine the tenderness of pork loin roast slices from 61 swine. Highly significant simple and multiple correlations were obtained among STE values for tenderness scores given by the panel. The correlations ranged

from .55 to .79 and indicate that this objective tenderness method can be effectively used to measure certain physical characteristics of pork tissue.

Pork loins from 96 pigs slaughtered at 75, 125, 175, 225 and 275 pounds live weight were tested for palatability and other characteristics. Muscle fiber diameter of <u>L. dorsi</u> increased linearly with slaughter weight. No clear-cut differences in patatability were found between pigs slaughtered at these widely-different ages and live weights.

Data on rates of change in electrical resistance obtained on 50 carcasses of reciprocal recurrent selection pigs indicated no significant relationship between electrical resistance changes post mortem and tenderness scores given by the panel. (AH a4-3)

### 2. Composition.

Analysis on data involving 97 hogs slaughtered at 50-pound weight intervals from 75 to 275 pounds is underway. This experiment was designed to evaluate live animal composition from ultrasonic measures of fat and thickness of eye muscle and to provide basic information concerning growth and fattening. Growth curves of total separable fat and lean indicate that the inflection point for these two carcass components occurs at about 175 pounds. The carcass weight added above 175 pounds is chiefly fat. Correlations between ultrasonic and actual backfat thickness were greatest in hogs of market weight (225 pounds). Multiple correlations obtained among ultrasonic fat thickness, recorded at the shoulder, loin and ham at each of the five weight intervals and total separable fat in pigs slaughtered at 175, 225 and 275 pounds, were highly significant (.73-.92) Ultrasonic fat measures recorded at 75 pounds were effective in predicting backfat thickness in pigs slaughtered at 175 pounds. For pigs slaughtered at 225 and 275 pounds, ultrasonic measures were not effective in predicting backfat thickness until pigs reached 175 pounds.

Research on the use of ultrasonics for evaluating live pigs has indicated that problems previously encountered with multiple fat layers in swine can be overcome by experience in the interpreting of pips as seen on the oscilloscope screen. Seventy-one animals of the high-fat and low-fat swine breeding study were examined ultrasonically for depth of backfat thickness over the ham and depth of lean over the last rib and ham. The thickness of the belly on the pigs at 10, 12, and 14 inches off the midline at the last rib was also recorded. These data are awaiting statistical analysis. The face of the fresh, trimmed ham was traced for fat, bone and muscle areas. These data

will be compared with ultrasonic estimates of fat depth and lean depth of the ham measured at locations 2, 4, 6 and 8 inches off the midline over the anterior portion of the right ham. (BS 3-34)

An analysis of data from 42 carcasses at the Oklahoma station indicated that one side could be used for measurement of specific gravity of the carcass and the ham, backfat and length. Removal of the ham from the carcass and subsequent separation into fat, lean and bone was more precise than removal and separation of the middle and shoulder. Carcass length was of no value if backfat was also considered in evaluating the carcass for leanness. The live probe indicated leanness as well  $(R^2 = .48)$  as backfat thickness  $(R^2 = .48)$  but not as well as backfat thickness and loin eye area at the 10th rib ( $R^2 = .70$ ). Other indicators of leanness equal to or better than this were: specific gravity of the ham  $(R^2 = .69)$ , specific gravity of the ham and loin eye area ( $R^2 = .79$ ), weight of lean and fat in hams ( $R^2 = .92$ ) and 8 different cross sections spaced from the ham shank to the 2nd thoracic vertebra (R2 ranged from .72 for ham shank to .89 for 3rd lumbar vertebra). The indices showed lower relationships to weight of separable lean and thus suggest they were more closely associated with leanness than with protein content of the lean. (AH al-8)

Live-animal measurements taken at the Wisconsin station on 830 animals at ages ranging from 84 to 154 days showed that measurements at 112 to 154 days may be used with a reasonable degree of reliability for predicting percent lean cuts at a slaughter weight of 205 and 225 pounds. Backfat thickness at the loin and body weight were the best two indicators of carcass merit. (AH al-10)

At Missouri, 6 live hog measurements were studied to estimate their relative value for predicting percent of live weight that was fat, five primal cuts, and adjusted loin equivalent. The measurements were backfat probe at (1) shoulder, (2) loin, (3) ham, (4) body length, (5) heart girth and (6) flank circumference. These measurements were most useful as indicators of percentage fat ( $R^2 = .51$  and .60). Significant breed differences in this respect were also observed. (AH al-6)

Plasma lipid levels were studied on 58 pigs of the fat-lean swine breeding study in the spring of 1962. No pronounced differences in average plasma lipid values were found between the high-fat, low-fat or control lines of pigs. However, pigs of Duroc breeding tended to have higher lipid values (135 mg. percent) than Yorkshire pigs (108 mg. percent). Female pigs within breed and line group appeared to possess lower lipid levels than those recorded for barrows of the same breed and line. Plasma lipid values were not strongly related to the degree of carcass fatness among the animals studied. These studies were conducted, in part, under contract with A. M. S. (AH a4-3)

### 3. Color.

Research on color in pork as influenced by heredity, sex, age, feeding, and mangement is being conducted as a PL 480 study with the institute of Animal Physiology and Nutrition, Laboratory for Animal Products, Polish Academy of Science, Warsaw, Poland. Methods and instruments that will be most useful in this study are being investigated. The first report describes trials using a simple photometric method involving a Pulfrich photometer (reflectometer) with Ulbricht ball and a reflectance spectometer. Forty pork samples were measured with the Pulfrich reflectometer at 7 wavelengths from 460 to 660 mm. In addition, reflectance values in the visible spectrum of 5-10 mm intervals within range of 460-660 mu were determined. With the spectrophotometer method the value of the dominant wavelength, saturation and lightness/brightness/color have been calculated. Values obtained with the Pulfrich reflectometer are being correlated with those obtained by the spectrometric method. The important factors of color are pigments and water-holding capacity. To determine if these factors are independent or dependent on each other, total pigments, myoglobin, and water-holding capacity were determined on 36 samples of pork. Statistically significant correlations at the one percent level were found between water-holding capacity and total pigment (r = .44), and water-holding capacity and myoglobin content (r = .46). (E21-AH-2, PL 480)

# C. Humane Slaughter

# 1. Cornell University.

Research is in progress at Cornell University to determine the physiological affect of immobilization with different percentages of carbon dioxide mixed with air or oxygen and to study the effects of carbon dioxide upon bleeding and carcass quality. Blood levels of 80 volume percent CO2, or more, were required to effect rapid immobilization. This level was reached with 68% CO2 combined with air or oxygen. Added oxygen will prolong the time an animal can maintain heart action. However, even at oxygen concentrations greater than atmospheric (25% vs. 21% in air) sheep cannot be maintained under 75% CO<sub>2</sub> for more than 15 to 20 minutes. Electroencephalograms from sheep and pigs showed similar changes following treatment with cyclopropane, nebutal or CO2. However, this could not be considered conclusive proof of the anesthetic action of CO2 without measurement of cerebrospinal fluid pressure or intracranial pressure, which may have been influencing factors. Muscle tissue from animals immobilized with CO2 and air was darker than tissue from animals immobilized with CO2 and oxygen, with the tissue color persisting for several days after slaughter. It may be desirable to have more  $O_2$  in a  $CO_2$  immobilization chamber than that supplied by air because of meat color and danger of killing animals. However, blood pressure is elevated with added oxygen and more hemorrhages were observed. (AH jl-1)

## 2. Beltsville, Maryland.

Investigations were continued into the effects of different methods of immobilization upon meat quality, and into the changes in skull development with advancing age. Seventy two swine were immobilized, half by electrical and half by mechanical means. No differences that could be attributed to the method of immobilization were found in the pH of the gracilis muscle or blood, blood glucose, blood creatinine, muscle glycogen, muscle lactic acid or observations on lungs, viscera and hams.

Studies were made on 28 sheep immobilized by captive bolt stunners to determine the effects upon arterial and venous blood pressure, heart rate and respiration. An electronic recording system was used. It was found that arterial blood increased to a peak within 15-25 seconds. Using a mean arterial blood pressure of 30 mm of mercury as an arbitrary point below which blood will not be pumped from the body by the heart it was found this point was reached in 3 minutes and 18 seconds to 7 minutes and 40 seconds. Pressure in the jugular vein also rose very high at impact, then gradually diminished. Heart rate increased at impact, reaching a peak in 15 to 25 seconds then gradually decreasing. Respiration ceased at time of impact where there was a solid blow to the cerebrum. At a period of from 6 to 25 seconds after impact there was a series of muscle spasms accompanied by a rapid irregular ventilation of the lungs. These spasms lasted from two to ten seconds, decreasing in duration. (AH j1-2)

## 3. University of Minnesota.

Investigation into the physiological effects of electrical immobilization and into the evaluation of pain are being conducted at the University of Minnesota. One field of study was the comparative effects of trans-occipital or trans-parietial stunning to that of the cranid-thorax or cranio-gluteal method. This latter procedure had been adapted by certain packing plants because of the absence of post-immobilization convulsions. Upon attempting to duplicate the conditions of the packing plants it was found that sheep stunned in the cranio-thorax or cranio-gluteal areas were instantly electrocuted. The mode of action of this type of stunning appears to be an arrest of the action of the nervous impulses traveling to the S-A node, after which some ectopic focus in the ventricles tries to maintain activity until advanced hypoxia prevents further action.

Studies were initiated into the complex problem of determining whether or not the animal feels pain upon being immobilized electrically. Using psychological and electrophysiological techniques, a method was developed to condition an animal to respond under certain stimulus. A conditional response was developed in the somatic (leg movement) and

the antonomic (respiration, blood pressures and heart rate) and the parameters measured by the electroencephalogram. The results indicate that a shock to the head of 10 M.A. magnitude is painful to the animal and that shocks of greater magnitude, such as those applied during stunning, should also be painful unless the animal is rendered unconscious by the activity of such a current. In conditioned animals somatic, autonomic and electroencephalgraphic responses were developed. This indicates that the stimulus used was painful to the animal and that the animal was not rendered unconscious by a current magnitude of less than 100 M.A. The conditioned somatic response was, however, erratic in its development under these conditions, which indicates that a degree of unconsciousness may have been developed but not to the extent that this stimulus would be acceptable for humane slaughter. In the second group of animals in which no condition responses were developed, indicates that a current of greater than 100 M.A. is sufficient to render the animal unconscious. In the third group of animals conditioned responses were developed in all measured parameters. This indicates that not only does a current of less than 100 M.A. magnitude not produce unconsciousness but that a retrograde amnesia does not develop as a result of this current. In a fourth group in which conditioned responses were not developed in any parameter, a current of greater than 100 M.A. magnitude not only results in a state of unconsciousness but also results in a retrograde amnesia of at least one minute duration.

In a study of pain pathways there is an indication that the "pain" pathways in sheep are limited to the ventro lateral quadrant of the spinal cord. The course of the fibers up the cord appear to exhibit some diffuseness, i.e., crossing over initially to the contralateral side and crossing back ipsilaterally after ascending 4—5 segments. This diffuseness, however, does not seem to equal that found in the pig by previous studies, but seems to be similar to that in the cat as illustrated by other investigators. (AH j1-3)

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# Humane Slaughter

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# INFECTIOUS AND NON-INFECTIOUS DISEASES OF CATTLE Animal Disease and Parasite Research Division, ARS

Problem. Losses from infectious and non-infectious diseases of cattle, other than those due to parasites, are estimated at approximately \$600 million annually. These losses materially increase costs of production and conversely decrease profits. In turn, they contribute to the cost of every purchase of meat, milk, and other cattle products to the consumer. Some of these diseases are transmissible to man. Determination and definition of the causes of cattle diseases, explorations for efficient methods of diagnosis, prevention, control, and when feasible, eradication, are the purposes of the research program.

#### USDA PROGRAM

The <u>Department</u> has a continuing long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of cattle. Research is being conducted on the diseases at the designated locations.

The <u>Federal</u> scientific effort devoted to research in this area totals 50.7 professional man-years. This effort is divided among sub-headings as follows:

Brucellosis, 2.3 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the universities of Maryland, Minnesota, and Wisconsin.

Paratuberculosis (Johne's Disease), 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

<u>Vibriosis</u>, 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreement with the New York State Veterinary College.

<u>Tuberculosis</u>, 4.6 at the National Animal Disease Laboratory, Ames, Iowa, and through contract with the Michigan State University.

Mucosal-Respiratory Disease-Complex, 1.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Indiana and Iowa Experiment Stations (mucosal) and the Colorado State University (respiratory - rhinotracheitis).

Mastitis, 6.2 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California.

Respiratory Disease (Shipping Fever), 5.0 at the National Animal Disease Laboratory, Ames, Iowa.

Leptospirosis, 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

<u>Infertility</u>, 3.0 other than vibriosis and trichomoniasis, at the National Animal Disease Laboratory, Ames, Iowa.

Epizootic Bovine Abortion, 3.4 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California.

Enteric Infections, 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Leukosis, 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Foot Rot, 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Keratitis, 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 73.2 professional manyears divided among subheadings as follows: Brucellosis 5.8, vibriosis 6.4, tuberculosis 0.8, mucosal-respiratory disease complex 6.2, mastitis 10, respiratory disease (shipping fever) 4.7, leptospirosis 7, infertility other than vibriosis and trichomoniasis 5.2, epizootic abortion 0.5, enteric infections 2.5, foot rot 0.8, infectious keratitis 2.1, eurolithiasis 1.3, other diseases (clostridia, pulmonary emphysema, white muscle disease, etc.) 17.8. Colorado, Connecticut, Georgia, Kansas, Maryland, Michigan, Minnesota, Ohio, Virginia, and Wisconsin are conducting studies on brucellosis. Seven southern and three northeastern states are working on vibriosis through two regional research projects (S-30, Diseases of Reproduction and NE-40, Pathology of Breeding Failure). Wisconsin and Michigan are conducting research on improving present tests for greater sensitivity in diagnosis of tuberculosis. Eight north central states are conducting research under NC-34 Mucosal Disease. Florida coordinates related work on an informal basis with the north central states on <u>mucosal-respiratory disease</u> <u>complex</u>. North central, northeastern, southern, and western regions are all conducting research on <u>mastitis</u>. Seven north central states and the Department are cooperating through regional research (NC-34, Shipping Fever of Cattle). Five southern and two northeastern states cooperate in regional research (S-30, Diseases of Reproduction, and NE-40, Pathology of Breeding Failure). Cooperative regional studies among four northeastern states (NE-40, Pathology of Breeding Failure) and four southern states (S-30, Diseases of Reproduction) are being made on infertility, other than vibriosis and trichomoniasis. California, in cooperation with the Department, is conducting research on epizootic abortion. Arizona, Connecticut, Colorado, and Missouri are studying bacteria and viruses found associated with intestinal infections of cattle, known as enteric infections. Colorado is conducting research on foot rot. Arizona, Kansas, Montana, Nebraska, Oklahoma, and Texas are conducting research on infectious keratitis. Five western states are cooperating in regional research (W-41, Urinary Calculi of Beef Cattle)- Urolithiasis. All regions are conducting some research on Other Diseases (clostridia, pulmonary emphysema, white muscle disease, etc.)

Industry and other organizations are engaged in the preparation of marketable biologic and pharmaceutical products. They conduct experimentation on vaccines and the formulation of chemical compounds and other medicinal substances for prevention and treatment of diseases of cattle. These companies generally will utilize their own facilities. Information gained in their research generally is confidential in nature as are expenditures for research and development. It is estimated that 80 professional man-years are devoted to this work by industry and other organizations.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

# A. Brucellosis

In 1961, at the Animal Disease Station, Beltsville, Maryland, the persistence and localization of Brucella abortus infection in 169 bovine females was determined. Major sites of localization were the udder and supermammary lymph glands. Infection was shown to persist up to 11 years in these locations.

After exposure to virulent <u>Brucella abortus</u> strain 2308, cattle produced two types of sero-agglutinins. These could be differentiated on their ability to withstand heat (65 C for 15 minutes). Subsequently the relative percentages and the persistence of the two types of seroagglutinins differed between the resistant cattle and the cattle that became infected. In general, resistant cattle produced a small percentage of heat stable sero-agglutinins which were limited to the first 70 days after exposure. However, cattle that became infected produced predominantly heat stable sero-agglutinins throughout the course of the disease.

Acidified plate test antigens were shown to be incapable of differentiating serologic reactions of infected cattle from those of Brucella resistant cattle. The tests were not selective but rather the results depended upon the magnitude of the titer to the standard sero-agglutination test and the pH of the antigen employed.

An experimental complement fixation test was less effective than the heat inactivation test or the standard sero-agglutination tests for the detection of infection in the immediate post-exposure period, except in a few animals that became infected but did not abort.

The potential value of infra-red spectroscopy as means of identifying and classifying typical and atypical strains of the genus Brucella was investigated further.

Five hundred and seventy-five apparently normal fetuses, asceptically obtained, were studied from a bacteriological point of view. Serological tests, direct cultures on several media, as well as inoculation into embryonating eggs and guinea pigs, showed that bacteria were generally present in embryos and that the fetal skin seemed to harbor more microorganisms than other tissues and organs in the fetus.

Brucella isolations were made from the fetuses of negative cows and from some having titers of 1:2000 and greater. Fetuses of every age group harbored Brucella, and organisms were isolated from about 4 per cent of all fetuses examined. More than half of the isolations were made from the skin. No Leptospira were isolated at any time.

Immunizing agents have been studied in an attempt to develop a satisfactory vaccine for adult cattle and over-age calves. Strains of Brucella of reduced infectivity for laboratory animals have been produced using (a) strepto-mycin-dependent strains, adapted to living on  $1000_{\rm u}$  per ml. for 1 year and then adapted to growing without the antibiotic, and (b) embryonating egg-adapted strains. The latter have been serially passed through embryonating eggs from 100 to 500 times, and are highly potent in eggs. A very mild disease is produced in laboratory animals, but following challenge they exhibited a high degree of resistance to infection with virulent forms of the organism.

In 1962, at the National Animal Disease Laboratory (NADL), Ames, Iowa, experiments were completed to determine immunogenic response of calves vaccinated at different ages with <u>Brucella abortus</u> Strain 19. Midway through their first gestation 69 vaccinated cattle and 22 nonvaccinated controls were exposed conjunctivally to approximately 7 x 10 cells of virulent <u>Brucella abortus</u> Strain 2308. Proof of infection was based upon isolation of the organism from one or more of the following sources: milk, dam's blood, uterine contents and fetus at parturition, or tissues obtained at necropsy.

Eight of 24 calves vaccinated at 4 months of age became infected and 5 aborted. Eight of 22 calves vaccinated at 6 months of age became infected and 5 aborted. Seven of 23 calves vaccinated at 8 months of age became infected and 6 aborted. Twenty of 22 nonvaccinated controls became infected and 15 aborted.

Although no significant differences in the degree of vaccinal immunity could be shown among the three groups of cattle vaccinated at different ages, vaccination at 4 months of age materially reduced the problem of persistent postvaccinal titers.

Natural infection with <u>Brucella abortus</u> in 2 bulls was studied over a period of several years. Serologic, bacteriologic, and histopathologic examinations were correlated with the clinical manifestations of the disease.

Seroagglutinin and semen plasma agglutinin titers persisted for at least 5 years. Brucella abortus was consistently isolated from the semen of both bulls throughout the course of the disease.

At necropsy Brucella abortus was isolated from the testes, epididymides, seminal vesicles and the ampullae of the ductus deferens.

Pathologic changes occurred throughout the genital tract. Granulomas, including sperm granulomas, were found in the epididymis of one bull.

The high percentage of ejaculates containing viable <u>Brucella abortus</u> emphasized the need for frequent bacteriologic examinations of semen for evidence of <u>Brucella abortus</u> infection in all bulls used for artificial insemination or natural service.

A modified Coombs' test for the detection of incomplete antibodies was evaluated on serum frozen and stored at dry ice temperature from a previous exposure of vaccinated and nonvaccinated cattle. The test failed to reveal the presence of any incomplete antibodies during the course of the disease.

The conglutinin-complement absorption test was also evaluated on the same lots of frozen and stored serum. Under the conditions and limitations of the experiment the conglutinin-complement absorption test appeared to have no advantage over the standard seroagglutination tube and plate tests or supplementary tests (heat inactivation, complement-fixation, or acidified plate antigens). Furthermore, the complexity of the test rendered it less adaptable for a routine diagnostic laboratory tool.

The entire stock culture collection was repacked and recataloged for the move to the new Ames laboratory, consequently all lyophilized cultures are more readily accessible for future research purposes.

A critical study of the tube agglutination test for the diagnosis of brucellosis in cattle has been inaugurated within the last few months. Sufficient data has not been obtained on any of the variable factors to submit results at this time, but considerable information has been accumulated. Perhaps a better testing procedure may not result from this investigation, but it is possible that some minor changes could be made, which might improve the test.

An attempt has been made to develop a vaccine satisfactory for adult animals and over-age calves, which will not produce a lasting titer. Streptomycin-adapted strains, grown with 20,000 micrograms per milliliter of the antibiotic over a period of time, have been used for guinea pig vaccination. These strains are not streptomycin-dependent, but have been modified in growing with the antibiotic to such an extent that vaccinal titers produced by them are low and transient.

Six embryonating egg-adapted strains of Brucella are being serially carried forward in eggs, one strain having been passed 626 times and others through a lesser number of transfers. Results of trials with egg-adapted Strain 19 (175th passage) and B.C. (600th passage) indicate that both strains have some value as immunizing agents.

Experimental work on the protective quality of cattle blood serum has been confined to further study of 106 vaccinated heifers, which have been tested at monthly intervals.

In 1961, at the University of Maryland under a cooperative agreement with the USDA, studies on Actomer were continued regardless of the toxic effect of the acetone in which it must be dissolved. Experiments, using Actomer in guinea pigs have shown the drug to be effective for Brucella, even in very dilute solutions.

In 1962, Actomer was further studied in relation to its solubility in non-toxic agents. Mice have been used for the <u>in vivo</u> experiments.

In 1961, at the University of Minnesota under a cooperative agreement with the USDA, the experimental work on the protective qualities of cattle blood serum against Brucella was confined to a study of the serum obtained from calves and heifers. Data has been accumulated to complete the results on more than 2000 animal sera.

A Brucella agglutinating component (11.7 S) has been isolated from the milk of 2 cows and appears to differ in several major respects from the brucella agglutinating macroglobulin (19 S) of blood serum.

Epidemiologic, serologic, and bacteriologic studies of Brucellosis "Problem Herds" have been of material assistance in establishing 7 brucellosis-free counties in Minnesota during the past 8 months.

A differential diagnostic test was developed which may be useful in differentiating "non-specific" and "specific" agglutinins for brucella in blood serum and this new test is now undergoing further evaluation.

In 200 herds with long histories of brucella infection, the plate sero-agglutination test failed to identify almost 60 percent of probably-infected cows. In 681 herds, without such a long-standing history of infection, the plate test failed to identify only 16 percent of probably-infected animals. In the problem herds, the combination of plate, tube and whey agglutination tests properly identified 40 of 46 animals proved bacteriologically to be infected.

Herds with consistently positive ring tests which fail to disclose reactors to the plate sero-agglutination test have also been investigated. In most cases, a single animal has been found to be causing the ring test reaction. All were identified by the combination of plate, tube and whey tests.

In 1962, at the University of Minnesota under a cooperative agreement, studies of nonspecific agglutinins (NSBA) in bovine milk were continued to develop improved methods of isolation and increase the yield for further characterization of the macroglobulins. Studies on the use of mercaptoethanol to detect macroglobulins (NSBA) in bovine milk were inaugurated using the procedures which were developed last year.

Studies on mercaptoethanol to detect macroglobulins (NSBA) in blood serum of cattle, which react to the tube sero-agglutination test at dilutions of 1:25 or greater, are being continued in the development of a new procedure which may be useful in the field.

A Modified Cream Ring Test has been developed as a result of studies during 1960-61 which showed that the present cream ring test may fail to detect brucella agglutinins in up to 50% of cream samples tested when such cream has partially deteriorated or soured prior to sampling. The new Modified Cream Ring Test is being evaluated in this laboratory and five field laboratories by comparison with epidemiologic, serologic, and cultural studies of animals in cream ring test positive and negative herds. Results to date appear to support the developmental work in which it was observed that the Modified Cream Ring test would detect up to 50% additional samples containing brucella agglutinins in those cream samples which were partially deteriorated or sour.

Studies of problem herds are continuing using nine serological tests of serum and milk supplemented by bacteriologic and fluorescent antibody studies to isolate or identify the organism from those animals which are slaughtered. With the aid of these procedures 20 counties in Minnesota have been certified as brucellosis-free, and the studies are progressing in approximately 50 other counties. It has been interesting to note that in the first 18 counties certified - some for several years - only 5 herds have been found to have reinfection.

Studies of the milk brucella ring test are continuing to obtain more information regarding optimum methods of storage, handling, and preserving composite milk samples collected for Babcock testing in the plants in order that optimum samples for BRT testing may be obtained from these composite samples. Studies are now in progress to determine the effect of different concentrations of mercuric chloride on brucella agglutinins in the milk of cows at an earlier stage of infection. Studies are being conducted using 1½ ml. and 2 ml. of milk along with the regular milk Brucella ring test. These are being accompanied by studies of herd size and the effect of the number of animals contributing to the pool of milk on the present sensitivity of the brucella ring test under Minnesota conditions. These studies are particularly pertinent since the size of Minnesota herds is increasing each year.

In 1961, the University of Wisconsin, under a cooperative agreement with the USDA, developed a culture system using guinea pig monocytes for the assay of toxicity and antigenicity of extracts isolated from cells of B. abortus. The basic characteristics of a strain of brucella phage, useful in taxonomic work, have been described.

In 1962, at the University of Wisconsin, in studies of 262 herds with long standing infection, 230 were freed of brucellosis by a combination of serologic tests. In these herds the combination of plate tube and whey agglutination tests properly identified 41 of 48 shedders. The tube test alone identified 40 and the plate test alone only 25. The discrepancy between plate and tube test results on infected animals could be reduced by half but not eliminated by incubating plate tests for 15 minutes.

Two hundred forty-nine herds with consistently positive ring tests but without reactors to the plate test have been studied. In most cases, a single animal has been shown by titration with pooled negative milk in a ring test, to be responsible. Of 49 such animals which were subjected to bacteriologic examination, 18 were shedders. All were properly identified by the combined whey and tube tests.

Fractions of <u>Brucella</u> <u>abortus</u> have been studied in a culture system, using guinea pig monocytes. Differences have been found in toxic and hypersensitive effects of various fractions.

A phenotypic morphological effect of brucellaphage on some strains of  $\underline{B}$ . abortus has been shown to be due to a carrier state.

### B. Paratuberculosis (Johne's Disease)

In 1961 research was continued at the Animal Disease and Parasite Research Division's Station at Auburn, Alabama, on a herd of cattle infected with Johne's disease which had been under study for 56 months. During this period, 94 animals have been culled and examined post-mortem. Neither the complement-fixation test nor the hemagglutination test can reliably distinguish between infected animals and non-infected animals as determined by postmortem results. Although the skin test is commonly used as a diagnostic test, results thus far obtained in this herd indicate that it is far from satisfactory.

An experiment designed to study the use of a vaccine against Johne's disease in sheep has been in progress 4 years. Postmortem examinations have been conducted on 25 vaccinates and 31 controls. A few typical small acid-fast bacilli were observed in the intestinal mucosa of 2 vaccinates and 1 vaccinate was destroyed in an advanced stage of the disease. Two controls died from Johne's disease, 8 others were destroyed in advanced stages of the disease, and 2 animals randomly selected were found to be moderately infected on post-mortem. From these results it appears that the vaccine has definite value as a control measure for Johne's disease.

Four rabbits were inoculated intravenously with Mycobacterium paratuberculosis obtained by digesting infected intestinal mucosa with trypsin. These rabbits all remained healthy and in 3 instances typical small acid-fast bacilli were observed in smears prepared from intestinal mucosa. However, no evidence of progressive Johne's disease was observed in any of the rabbits.

Primary cultivation of  $\underline{M}$ . paratuberculosis from infected tissues was best accomplished by trypsin digestion of intestinal mucosa, followed by treatment with NaOH, and inoculation of lymph gland-egg yolk medium. It was found that the retarded growth exhibited by organisms from infected lymph glands might have been due to antibodies present in the lymph gland tissue. Growth of a recently isolated strain of  $\underline{M}$ . paratuberculosis was not inhibited by 10,000 units of penicillin when the organisms were seeded on lymph glandegg yolk medium, but there was no growth on lymph gland medium.

Protoplasm from M. paratuberculosis was used as an antigen for antibody production and as an antigen in precipitin tests. When it was used to elicit antibodies it produced a nodule at the site of inoculation and the animal produced precipitating antibodies within 2 weeks, but it did not react to intradermic johnin. When the protoplasm was used as a precipitin antigen, it reacted strongly with the serum from a sheep with clinical Johne's disease, and weakly with sera from sheep vaccinated with whole M. paratuberculosis or exposed to infection with Johne's disease but not showing clinical symptoms.

In 1962 the work on paratuberculosis was moved to the National Animal Disease Laboratory, Ames, Iowa. The herd of cattle ranging in numbers from 161-195 animals in which Johne's disease has been a severe economic problem has been under study for 68 months. During this period, 119 animals have been culled and examined postmortem. One of the 25 that were culled during the past 12 months showed clinical signs of Johne's disease. Neither the complement-fixation test nor the hemagglutination test can reliably distinguish between infected animals and noninfected animals as determined by postmortem results because M. paratuberculosis were found in 25% of the cattle negative to the complement-fixation test and in 36% of the cattle negative to the hemmaglutination test. The skin test using intradermic johnin is commonly used as a diagnostic test, however, results thus far obtained with intradermic johnin in this herd indicates that it needs much improvement because M. paratuberculosis were found in 40% of the non-reactors examined postmortem.

Two experimental skin test products were also tried on this herd. One was almost equal to standard johnin in potency and the other was about 2/3 as potent.

Acid-fast bacilli were demonstrated in the lungs, spleens, kidneys and livers of rabbits for at least eight weeks after they had been injected intravenously with either killed or live M. paratuberculosis. Therefore, it is questionable whether a true infection was established in rabbits with the live microorganisms.

Continuing studies of various types of media showed that lymph node-egg yolk medium is better than simple egg yolk or lymph node media for primary isolation of  $\underline{M}$ . paratuberculosis. A medium prepared from infected intestinal mucosa produced luxuriant growth of  $\underline{M}$ . paratuberculosis after prolonged incubation.

Rabbits and guinea pigs were vaccinated with a protoplasmic antigen and the controls with heat-killed organisms. They were skin tested with johnin and tuberculin. The skin reactions due to protoplasmic vaccine were small or absent. Those due to vaccination with heat-killed organisms were very large. Gel-precipitin tests with sera from protoplasm vaccinated rabbits produced only one definite precipitation zone. No precipitation zones were produced by sera from rabbits vaccinated with heat-killed organisms.

A new proteolytic enzyme (X-108) prepared by American Cyanamid Company, was found to be better than trypsin for digestion of tissues infected with Mycobacterium paratuberculosis and liberation of the bacilli.

#### C. Vibriosis

In 1961 the studies on vibriosis were in progress at the Animal Disease Station in Beltsville, Maryland. The morphology and biochemistry of bovine Vibrio strains isolated from 87 cattle in a survey of 56 herds were studied. The V. fetus strains (pathogenic) were separated into 2 major types and a subtype of Type 1, by colony dissociation and biochemical reactions. Type 1 strains grew fastidiously and growth was enhanced by the addition of glutathione and sodium thioglycollate to the medium. Both smooth and smooth-cut glass colonies were observed in primary cultures. Type 2 was characterized by more abundant growth and numerous stable variant colonies. Both smooth and rough colonies were observed in primary cultures. Subtype 1 strains grew moderately in culture mediums. Cut glass colonies were observed only at primary isolation. Vibrio bubulus strains (nonpathogenic) grew well on blood agar but they did not adapt easily to Albimi agar. Smooth and granular colonies were observed in primary cultures. Biochemical tests which were made on cultures of smooth and variant colonial forms failed to show any change in activity due to dissociation.

Bulls that were infected with Type 1 and Subtype 1 strains of  $\underline{V}$ . fetus infected all heifers bred to them. The infected heifers became repeat breeders. However, bulls that were infected with Type 2  $\underline{V}$ . fetus infected only 1 of 12 heifers by breeding, 11 of which required only  $\underline{1.4}$  services per pregnancy. Vibrio which was biochemically similar to the Type 2 exposure strain was isolated from both the vagina and the rectum of 1 heifer. This heifer was apparently not pregnant at slaughter 21 days after service.

A bull that was infected with Type 1 <u>V</u>. <u>fetus</u> infected 12 heifers at their first service. Uterine infection was found in 9 of these heifers at necropsy. A neutrophilic and lymphocytic infiltration of the endometrium was found on histopathologic examination. In contrast, neither infection nor histopathologic changes were observed in 5 virgin heifers that were necropsied at intervals during the estrus cycle. Seven heifers that were bred to a non-infected bull became pregnant, but all were free of histopathologic changes at necropsy.

Cultures of the three types of  $\underline{V}$ . <u>fetus</u> and fecal samples from cows infected with Type 2  $\underline{V}$ . <u>fetus</u>, were given <u>per orum</u> to noninfected cows. Isolations of Type 2  $\underline{V}$ . <u>fetus</u> were made from 21 of  $\underline{60}$  post-exposure fecal samples. At necropsy, Type 2  $\underline{V}$ . <u>fetus</u> was isolated from the intestinal tract from 3 of the 7 animals and also from the bile of one cow.

In 1962, most of the work on vibriosis was done at the National Animal Disease Laboratory at Ames, Iowa. Comparative infectivity studies of 20 strains of V. fetus included 12 strains of type 1, 5 strains of subtype 1, and 3 strains of type 2. Only type 2 strains consistently infected the gall bladder and

duodenum of mice, guinea pigs, and rabbits. These results compare favorably with experiments in cattle in which only type 2  $\underline{V}$ . fetus was recovered from the gall bladder and/or duodenum. It was concluded, therefore, that the digestive organs may harbor virulent  $\underline{V}$ . fetus intestinalis and remain the source of infection in cases of sporadic abortion in cattle.

Chemical, serological, and toxicological analyses were made on cell wall, intracellular, and extracellular fractions of the organism. The cell wall is composed of a complex group of amino acids characteristic of Gram negative organisms. At least 3 antigen-antibody complexes were found in agar-gel diffusion tests. Only the intracellular fraction was lethal for mice in the preliminary studies made.

A liquid culture medium was developed in cooperation with the chemical and physical investigations which improved the yield of  $\underline{V}$ . fetus cells from 0.5 gram to 2.0 grams per liter of culture medium.

In 1961, at the New York State Veterinary College, Ithaca, New York, under a cooperative agreement with the USDA, experiments were conducted to determine if vibriosis was spread by the methods commonly used for semen collection in an artificial insemination stud. Thirty-one bulls were used - half were collected in the usual manner and half with a so-called strict collection technic with no false mounts. Over a 2-year period, no new cases of vibriosis have been detected in the strict collection group, whereas 2 new cases were observed in the regular group. The strict collection method appears to minimize the chances of spread of the infection.

Eight bulls were treated by the application of a 1% furoxone ointment to the penis and sheath. Ten bulls were treated with the same ointment plus a 1% furaltadone solution in a series of three treatments at 47-72 hour intervals. Seven bulls, following treatment, were found to be free of  $\underline{V}$ . fetus at the end of the experiment. The procedures are being continued using the same chemicals in a more absorbable base.

Attempts to find more suitable culture media and technics are being continued. Albamycin has proved highly useful to suppress the growth of B. proteus. The filtration technic of Plummer and associates is being used regularly with highly encouraging results. In the filtered material only 2 percent of the cultures were overgrown, whereas 73 percent were overgrown when non-filtered material was used.

#### D. Tuberculosis

In 1961, research workers at the ADP Station at Auburn, Alabama, reported finding a herd consisting of 200 cattle, all thought to be free of Johne's disease and tuberculosis, disclosed 24 animals with reactions to tuberculin and/or johnin. Three of these were calves. The 21 adults were slaughtered, examined post-mortem for tuberculosis (none was found) and material was obtained from each animal which was processed and inoculated onto medium and

into small laboratory animals. None of the laboratory animals developed lesions of tuberculosis or reacted to the tuberculin test. However, acid-fast bacilli were obtained from cultures from 3 animals. These have not been identified. The ileocecal valves and adjacent regions of the intestinal tract were examined for the presence of bacilli resembling Mycobacterium paratuber-culosis. Such organisms were found in the intestines of 3 animals. Material from these three specimens has also been cultured, but this work has not yet been completed. Titers of 1/10 or more were observed in serums of 15 animals prior to skin testing and this titer remained fairly constant each week for four weeks.

In 1962, at the National Animal Disease Laboratory, at Ames, Iowa, data were collected on the response in cattle to intradermal cervical tests with different strengths of tuberculin given in 0.1 ml. amounts. The NADL herd was tested on the assumption that the animals were free of tuberculosis based on a history of no tuberculosis in the herd for over 25 years.

The skin reactions were recorded in terms of increases over and above the original skin thickness. The reactions ranged from (pp) a pin point disturbance through (p) 2 mm. increase, (p) 3 mm. increase, (p) 4-5 mm. increase, and (p) 5-6 mm. increase. In general, a p or greater is considered a significant reaction.

The data showed that when the animals were tested with ARS tuberculin diluted to 10% of the regular strength supplied for use in the field, no animals had greater than pp reactions at 48 hours and only 3 of 120 animals had p responses at 72 hours. In contrast, testing the same animals with regular strength tuberculin resulted in 14 p and 3 p reactions at 48 hours. Ten p and 3 p reactions were found at 72 hours. Testing 69 of the animals with tuberculin concentrated to 4 times the regular strength did not give results differing greatly from those occurring with regular tuberculin, although 1 p and 1 p reaction was observed at 48 hours and 2 p reactions at 72 hours.

The evidence indicates that a less concentrated tuberculin results in fewer skin reactions of all sizes in a herd apparently free of tuberculosis.

As part of a cooperative research problem with Chemical and Physical Investigations, a comparison of the antigens in BCG culture filtrate, water extracts of BCG, and preparations from unextracted and extracted BCG subsequently broken under mechanical pressure, have been compared. By gel-precipitin techniques, common antigens were demonstrated in all of the preparations. The cracked cell preparations had an additional precipitin line not demonstrated with the other antigens.

In 1961 and 1962, at Michigan State University, East Lansing, under a contract with the USDA, investigations were continued on the cause or causes of nogross-lesion tuberculin reactors and to improve methods of diagnosis of bovine

tuberculosis. All acid-fast microorganisms isolated by various methods from animal tissues, including skin lesions, and soil samples were identified insofar as possible by selected morphologic, cytochemical, infectivity and sensitizing characteristics. Emphasis was placed upon there being no single test which conclusively identifies any one species of the known mycobacteria. For instance, Mycobacterium tuberculosis produces nicotinic acid and this is perhaps the most dependable and widely used characteristic for confirmation. However, M. microti and M. ulcerans are also positive. The demonstration of the tubercle bacilli in pathological material is the only sure method for diagnosis.

A wide spectrum of acid-fast organisms, including many atypicals with varying degrees of virulence and all reacting to some extent to mammalian, avian and to various purified protein derivatives (PPD), were found in the tissues of tubercular positive cattle. To date mycobacteria isolated from 263 cases have been tentatively identified. Of these, 81 were from bovine tissues, other than skin lesions, 22 being M. bovis and 2 M. avian; 85 from skin lesions, 5 from bovine semen specimens which may have become contaminated at time of collection, 96 from swine lymph nodes, 1 being M. bovis, 35 from soil and barn samples, 5 from feed samples and 1 each of guppy and mink origin. In some instances a number of isolants from a single case were tested.

The isolation of many acid-fast organisms, which may be incorrectly called "atypicals", from tuberculin positive cattle that are infective to laboratory animals reveals for the first time a new group of organisms that must be fully evaluated by large animal studies. Infectivity, sensitivity, transmissibility and serologic and immunologic behaviors must be investigated before these organisms can be cataloged and their importance in disease control evaluated.

Research findings that the procedures used for the isolation of acid-fast organisms from samples containing low populations is applicable for the isolations from samples containing high populations. However, the reverse is not true. A total of 121 cases consisting of tissue samples were each processed by two methods, the results reported show there were 22 positives isolated by the sodium hydroxide method and 44 isolates by the pentane-digest method.

The difficulties encountered in the classification of acid-fast organisms isolated from gross lesion and no-gross-lesion reactors and the increasing interest in the pathogenic significance of unclassified acid-fast bacteria have led to the initiation of research concerned with the nature of proteins in and produced by various types of acid-fast organisms. The primary purpose of current studies is to develop procedures to produce more specific sensitins for the identification and differentiation of the various mycobacteria and mycobacterial infections in animals.

### E. Mucosal-Respiratory Disease-Complex.

In 1961 the studies conducted at Purdue University, Lafayette, Indiana, under a cooperative agreement with the USDA on reciprocal cross protection tests in calves indicated the Indiana virus diarrhea agent (IVD), the Oregon virus diarrhea agent (OVD), and the Indiana mucosal disease agent (IMD) were immunologically identical. Attempts were made to neutralize the cytopathogenic OVD agent with 14 different types of antiserum. Seven types related to the mucosal-disease virus-diarrhea complex did neutralize this agent the others did not. Various determinations were made of the properties of the OVD agent. It was found that it contained little or no lipid, was between 50 and 100 millimicrons in size, did not cause hemadsorption of guinea pig erythrocytes, and was destroyed by 56 C. in approximately 30 minutes.

The OVD agent did not readily adapt to embryonated eggs, suckling mice, or rats. Sheep fetuses propagated the agent in low concentrations following in utero inoculation.

A specific pathogen free cattle herd was originated by performing 22 caesarean sections and raising the calves in an isolated environment.

Other results indicated that several of the mucosal disease and virus diarrhea agents are related. This provides evidence that these agents are actually part of the etiology of the diseases and indicates that virus diarrhea and mucosal disease may be different clinical manifestations of the same disease. This hypothesis will be difficult to prove until means are devised to produce the typical clinical syndromes.

Calves contact-exposed to inoculated sheep developed signs of disease. When these calves were challenged with virulent blood they developed typical signs of experimental virus diarrhea. Blood collected from the sheep during the period of leukopenia was infective for calves, indicating that viremia existed at the time sheep-calf contact was made. Calves infected with the sheep blood resisted challenge with virulent bovine blood.

Attempts to demonstrate calf-to-calf contact transmission were unsuccessful. Calves exposed to virus by the nasal-oral route did not develop signs of disease. When these calves, and calves in contact exposure with them, were challenged with bovine virus they all reacted in a typical manner. The contact calf exposed to an intravenously inoculated pen-mate did not develop signs of disease. It was fully susceptible when later challenged.

In 1962, at Purdue University under a cooperative agreement, investigations of field outbreaks in the mucosal disease complex continue to reveal a sporadic distribution of both MD and VD. Chronic diarrheal syndromes in feeder calves continue to pose a diagnostic problem for practicing veterinarians in Indiana.

There is usually no history of an acute disease syndrome (such as acute virus diarrhea) in these chronically affected herds. Invariably only a portion of the herd is chronically affected. It is probable that affected herds experienced a previous, mild infection of short duration, although this information is not always obtained from the herd history. In these cases, diagnosis of VD-MD complex is made on a basis of history (when available), clinical observation, and serology. Serological diagnosis is based on high titers of neutralizing antibody against the type strain of VD virus--Oregon C24v.

In three trials, intravenously inoculated sheep were placed in direct pencontact with susceptible calves. Control transmission tests with calves were performed in which orally and parenterally inoculated calves were placed in direct contact with other susceptible calves. Bovine blood was used as stock virus. In no case was contact infection established in calves exposed to infected sheep. When later challenged, these calves were fully susceptible to the virus.

Calves in direct contact with other calves given virus either naso-orally or parenterally failed to show signs of experimental infection. Subsequent challenge with stock virus showed that contact-exposed calves were fully susceptible.

Although viremia was demonstrated in sheep in two trials, exposure of calves to them at this time did not result in contact infection.

Tissue culture systems employing bovine and ovine thyroid tissues were developed. Preliminary studies indicate that Oregon (C24v) and Nebraska (M-833) agents replicate and produce cytopathic effects in monolayers of ovine thyroid cells. This cell system is applicable for virus neutralization tests. Studies are in progress to determine the susceptibility of these cell systems to virus isolates that fail to elicit cytopathic effects in other cell lines.

Procurement of over 40 calves by Caesarean section furthered development of the Specific Pathogen-Free (SPF) cattle herd. The opportunity was taken to study the possible presence of bacteria and viruses in the <u>in utero</u> environment. Bacteriological examination of the fetus, umbilical cord blood, and extra-embryonic fluids showed that these calves were apparently free of bacteria at time of section. Study of developmental changes in blood cellular elements and serum proteins were made at birth and early in post-natal life. Methods for rearing of these colostrum-deprived calves were refined.

Sterile swabs were used to obtain specimens from the amino-allantoic fluids, fetal, skin, anus, and nose. Samples of placental blood have been obtained from the umbilical cord. All samples were subjected to anaerobic and aerobic culture conditions. Blood agar and thioglycollate media were inoculated and incubated at 37 C for 48 hours.

Bacteria were present in cultures from four of 29 calves. Two isolates of non-pathogenic organisms were from the nasal area. In both calves the specimens were not obtained until after respirations had been initiated and the isolates were considered to be air borne contaminates. A gram positive cocci from the amniotic fluids and a coliform organism were isolated from two other calves. Possible contamination of a calf was known to result from an accidental perforation of the maternal intestinal tract. The efficiency of the bacteriological procedures was demonstrated by the isolation of bacteria from the nose, skin, and anal region of this calf.

The bovine placenta effectively interferes with the transfer of maternal antibodies to the fetus. Passive immunity is provided for the newborn calf by enteric absorption of colostral antibodies. As the Caesarean derived calves were deprived of colostrum, this provided an opportunity to evaluate the functional development of the reticulo-endothelial system of the calf. Serum or plasma samples were collected from the blood samples obtained for hematology. Electrophoretic analysis of the serum (plasma) proteins are being utilized to give a qualitative evaluation of the various components, which are then quantitated by nitrogen determinations. This work is in progress.

In 1961, at the Iowa State University, Ames, Iowa, under a cooperative agreement with the USDA, it was found that, with the exception of the combination of the Sanders and PPLO agents and the delayed combination of Sanders and Nebraska MD agents, the series of experiments involving a combination of agents of the mucosal disease viral diarrhea complex produced a response which closely approaches the field syndrome than if these agents are inoculated by themselves. On the basis of these preliminary experiments one can hypothesize that mucosal disease as it is seen in the field probably is the result of an infection by a combination of specific agents plus possibly other factors. The proof or disproof of this hypothesis awaits further trials.

A definite, repeatable syndrome can be reproduced in calves by the inoculation of the Sanders agent. Furthermore, it has been observed that this agent can be serially passed from one calf to another with the typical syndrome appearing at each passage. Swine and goats showed no conclusive response to the Sanders agent. Sheep appear to be susceptible to the Sanders agent and develop a syndrome milder but similar to that seen in calves. Present information indicates that mice, guinea pigs and chicken embryos show no evidence of susceptibility to the Sanders agent.

In 1962 workers at the Iowa State University reported a viral agent was isolated from a calf from the Colglazier herd showing typical signs of mucosal disease. This agent was recovered from deep scrapings of involved Peyer's patches. Isolation was accomplished by four passages through primary cell cultures of bovine kidney and testicle cells. The virus produces distinct cytopathic effects in primary cell cultures of bovine origin. A plaque method was devised enabling a highly accurate titration method. Using this method,

cross neutralization tests against known strains of virus diarrhea agents were done. Results of these tests indicated the absence of any serological relationship between the newly isolated agent and two reference VDV virus antisera produced in rabbits. The virus is not pathogenic for mice by any route. There is indication that a disease syndrome is produced in cattle, although insufficient trials will not permit us to make definite claims.

Preliminary trials using fluorescent antibody techniques to detect specific viral antigen in cell cultures and frozen sections have been done. It appears that specific antigen can be localized in cell cultures. We are presently trying to improve our methods with the intention of applying this method to diagnosis and basic research including the study of non-cytopathic strains and their serological relationships.

Attempts to isolate enteroviruses from 128 animals located in eight different herds and ranging in age from 3 days to approximately 5 years have been negative. Serum samples collected from 37 animals either affected with mucosal disease or located in infected herds have failed to neutralize the Nebraska mucosal disease agent. Serum samples from experimentally infected calves or hyperimmunized rabbits have shown that only the Nebraska and the North Dakota agent antiserum are capable of neutralizing the Nebraska mucosal disease agent. Antiserum against the Sanders, Merrell, Indiana virus Diarrhea, C-80-K and C-24-V agents fails to neutralize the Nebraska mucosal disease agent.

Many combinations of agents isolated from the mucosal disease-virus diarrhea complex have been used in an attempt to reproduce a typical case of mucosal disease terminating in death. However, our experimental trials have not been successful in this regard.

In 1961, the University of California, at Davis, California, in cooperation with the USDA, reported a filterable agent, which appears to be viral in nature, has been isolated from two different cows out of some 30 cows clinically diagnosed as suffering from bovine lymphosarcoma.

Cattle experimentally infected with infectious bovine rhinotracheitis (IBR) virus showed detectable antibodies after 44 months. Studies to date have been encouraging enough that a CF serology test may be devised to detect IBR antibodies.

A preliminary survey by means of serum neutralization in tissue culture has been initiated in the comparison of some cattle diseases of the United States to those which exist in Germany. Attempt to isolate the virus diarrhea virus of cattle in tissue culture from materials obtained from clinically diagnosed cases of virus diarrhea was not successful. Studies with the "viral" agent of bovine abortion were made in regard to its behavior in young calves and possible placental transmission.

In 1961 the Colorado State University, at Fort Collins, under a cooperative agreement with the USDA, reported that twenty cattle were continued on an experiment in a large animal virus isolation laboratory at Fort Collins to determine the rate of decline of antibody titer following inoculation with virulent virus of infectious bovine rhinotracheitis. During the year 3 animals showed 1000 fold reductions of antibody titers after 7 to 10 months. Their immunity was challenged by intratracheal inoculation of virulent virus. One developed typical symptoms of the disease, 2 showed increase in body temperature only, and 3 showed anamnestic response by augmentation of titers. The remaining 17, with the exception of the 4 controls, still had high titers 18 months after intratracheal, intramuscular, or intravenous inoculations. Seemingly there is no significant difference in titers as a result of different route of inoculation.

By the use of the fluorescent antigen-antibody technique, a study of virus infected bovine kidney cells in a tissue culture system showed no evidence of virus aggregation forming the inclusion body. Materials were received from 48 outbreaks for virus isolations from 11 States and from 280 cases for serum neutralization tests.

In 1962 studies were continued at the Colorado State University under the cooperative agreement, and during the year the serum neutralizing titers of cattle which are kept in the isolation units, did not show significant reduction. There was no evidence of difference in neutralizing antibody level between the two groups of animals infected intratracheally and those infected intramuscularly.

In a study of abortion of cows in relation to IBR infection, the isolation of IBR virus from aborted fetuses was the first stage of the work. Field reports of abortions were investigated. Fetuses were collected, and different organs of the fetus were used for isolation of IBR virus. The virus was isolated from the lung tissue of the fetus only. If more fetuses could be obtained it might be possible to isolate the virus from other organs.

In studying IBR epizootics in ranch cattle, fifty animals (47 deer from 1 to 6 years of age, and 3 elk from 1 to 2 years of age) were gathered together for testing. Blood samples from these animals were collected. These animals will be challenged or infected with IBR virus to determine their susceptibility and what sign(s) of sickness, if any, are manifested. Since deer, elk, and cattle cohabitate in pasture and range areas, the susceptibility of deer and elk to IBR may be an important factor in determining transmission of the disease.

In 1962, at the National Animal Disease Laboratory at Ames, Iowa, naturally occurring cases of BVD or MD have been studied in the NADL herd from which one agent was recovered and in one farm herd from which 4 probably identical agents were recovered. Inoculation of the NADL agent into an experimental animal was studied for development of clinical signs, blood changes, virus recovery, and production of neutralizing antibody. Periodic bleedings have

been made from the NADL herd as well as from the farm herd. Isolation of additional agents from the respective herds was attempted by making rectal swabs and leucocyte cultures from apparently normal cattle. This resulted in the isolation of an agent from 1 out of 5 rectal swabs taken in the farm herd and from 12 out of 19 rectal swabs representing all cattle in one NADL barn. In addition, 5 isolates were obtained from leucocyte cultures out of the 19 apparently normal cattle housed in the NADL barn.

Virus neutralization studies indicate that the agents isolated from rectal swabs are antigenically different from those isolated by leucocyte cultures. They also appear distinct on the basis of rapidity and type of CPE produced in tissue cultures.

Five viral agents identified with bovine virus diarrhea (BVD) or mucosal disease (MD) have been obtained from other laboratories for storage in the repository and subsequent use as reference material. The Oregon virus diarrhea agent (C24V) has been selected as the prototype since it is the one most commonly used in other laboratories.

A bovine kidney cell line designated National Laboratory Bovine Kidney (NLBK) was developed as a laboratory tool. It appears to be very useful because it is susceptible to the prototype virus as well as several other viruses of bovine origin.

#### F. Mastitis

In 1961 research was continued at the Animal Disease Station at Beltsville, Maryland, on the development of a medium for the titration of lactenin. Beef infusion, when added to a medium containing casin hydrolysate, vitamins, glucose, and various salts, permitted the growth of Streptococcus pyogenes. The growth factor will pass through a cellophane membrane, will migrate to the cathode chamber in an electrodialysis cell and is stabile to autoclaving or concentration at 80 C. in a flash evaporator. The growth factor is not associated with carbohydrates or minerals present in the ash. Several amino acids, purines and a pyrimidine have been tentatively identified by chromatographic methods but have not been related, as yet, to the growth factor.

In 1962, at the National Animal Disease Laboratory, Ames, Iowa, research on mastitis was continued on the study of factors in milk which inhibit the growth of Streptococcus agalactiae. The inhibitory activity of milk was not affected by preparing whey by treatment with acid or rennin and centrifuging the wheys at speeds up to 17,000 rpm (34,800 X C). No loss in inhibitory activity was caused by dialyzing whey against de-ionized water or a 0.02 M phosphate buffer of pH 7.0 for 48 hours at 5°C. However, electro-dialyzing whey at room temperature for 7 hours at constant current (100 m.a.) resulted in a decrease in activity of at least 50 percent.

When milk from infected quarters was titrated raw, the dose-response curve typical for the inhibitory activity of raw milk from non-infected quarters was altered because of increased acid production. This increase in acid production was eliminated by pasteurizing the milk from the infected quarters before titration.

Research was also continued on the development of a culture medium of defined composition to grow streptococci of serological Groups A and B for use in assaying the growth inhibitory factors in milk. A culture medium, composed entirely of known constituents except for 1 mg per milliliter of crude egg albumin will support good growth of <u>Streptococcus pyogenes</u>. Washed cell suspensions which were grown in Difco brain-heart-infusion broth were used as a source of inoculum. Purified egg white fractions such as conalbumin, ovalbumin and perhaps lysozyme will substitute for the crude egg albumin. This is the same basic medium used for microbiological assay of amino acids and is described in Cornell Experiment Station Bulletin No. 337, 1955.

The same medium without the egg albumin, supported good growth of several cultures of <u>Streptococcus</u> <u>agalactiae</u>. These organisms are less fastidious than the group A cultures and will grow in a medium in which all components are known.

Growth of these two groups of organisms can be measured with speed and accuracy by titration of the acid formed. Both groups converted 90 or more per cent of the glucose fermented to lactic acid.

Four strains of <u>Str. agalactiae</u> were shown by analysis of their fermentation products to be homofermentative lactic acid bacteria.

In 1961, the University of California, at Davis, in cooperation with the USDA, in studies in a limited number of animals, the following results may be of significant value for future studies on the big and important research problem of mastitis.

A small number of <u>Aerobacter aerogenes</u> may induce clinical mastitis in a normal mammary gland. Repeated small doses did not lead to an intensification of the clinical response but may have contributed to development of an infection of 20 days duration.

A pre-existing chronic inflammation caused by <u>Ps. aeruginosa</u> gave some protection against development of an acute clinical phase of mastitis following the introduction of 4,000,000 <u>A. aerogenes</u>. A persistent infection was not established for the longest period of residence in any gland was 9 days.

In an attempt to counteract  $\underline{A}$ .  $\underline{aerogenes}$  infection, the inoculation of extremely large numbers of micrococci led to a high and somewhat persistent level of leukocytosis accompanied by swelling and clots. This was followed in 5 days by disappearance of the  $\underline{A}$ .  $\underline{aerogenes}$  infection, and on the 6th day the micrococci disappeared.

The introduction of approximately 1 million dead A. aerogenes organisms into normal lactating mammary quarters induced a leukocytosis of 4 days duration with the peak number of cells in foremilk reaching 4.0 to 5.0 million/ml. Viable organism in the same dose level stimulated cellular responses in 8½ hours of 60.0 million cells/ml and in addition swelling of the gland was observed for 3½ days and rectal temperature reached 106.0°F at 8½ but quickly returned to normal. During the period of persistence of the A. aerogenes within the gland a see-saw pattern of cellular activity was seen with two or more peaks of considerable magnitude followed immediately by a rapid fall in cell numbers. Presence of A. aerogenes in the milk was demonstrated mostly only by incubation of the milk before culturing on the surface of blood agar plates.

The information gained from other limited clinical, bacterial, and chemical studies on mastitis points to the value of gathering data during the early phases of the response to bacterial infection in the mammary gland in order to better understand the natural mechanisms for defense of the total animal. The response of the animal to infection is immediate and, therefore, future studies should include examinations at frequent intervals during the first few hours.

In 1962, the University of California, cooperatively with the USDA, studied a culture of Aerobacter aerogenes originally isolated from the bovine udder in 1959 which was employed in the production of experimental mastitis. It was demonstrated that less than 100 organisms, when introduced into the teat and gland cistern of an absolutely normal lactating mammary gland, are capable of inducing in 10-15 hours an acute mastitis with an associated systemic reaction. It was also clearly demonstrated that a pre-existing leukocytosis, even of low order, has significant protective benefits. Systemic signs, such as elevated body temperature and anorexia, and local signs, such as detectable swelling of the inoculated gland, are prevented from developing when leukocytes are present in the udder secretion at the time of introduction of the culture. The inoculum, however, causes an increase in exudation of neutrophils so that for several milkings the cell count/ml. is increased significantly, although visible signs such as clots in the milk may not occur.

The experiments reported here strongly indicate that mammary glands must be completely free of even a minimal inflammatory reaction before coliform-type organisms are able to produce acute mastitis. Patency of teat canal is also involved. The most apt to have patent streak canals are the older animals and since the older animals most regularly have a leukocytosis from repeated udder stress, such cows at the same time are protected against coliform mastitis. Application of extensive intramammary therapy to such cows may produce complete freedom of such glands from leukocytosis and render the animals susceptible to coliform mastitis.

# G. Shipping Fever

In 1961, at the Animal Disease Station, Beltsville, Maryland, as a continuation of studies on the etiology and transmission of shipping fever, 3 Holstein calves were injected intramuscularly with live para-influenza 3 virus, three with killed Pasteurella multocida and P. haemolytica, and three with a combination of the live virus and killed Pasteurella spp. Para-influenza was not transmitted from the injected calves to contact controls as determined by hemagglutination-inhibition test and attempts at virus isolation.

The 9 injected and 5 control Holstein calves were assembled in a feed lot with 27 Angus calves, of which several were showing clinical signs of shipping fever. P. multocida, P. haemolytica, and para-influenza 3 virus were transmitted to the Holstein calves by contact. P. multocida, P. haemolytica and para-influenza 3 virus were isolated from 8, 13, and 9 Holstein calves respectively. The injection of the above three agents before contact exposure could not be adequately evaluated under the conditions of the experiment.

In 1962, at the National Animal Disease Station, Ames, Iowa, fundamental studies on the nutrition and metabolism of Pasteurella sp., believed to have etiological relationship to shipping fever, are in progress. Blood agar base containing hemin was the best medium tested for the enumeration of P. multocida and P. hemolytica, producing the highest, least variable counts, while gelatin-saline was the best diluent for counting procedures.

Studies designed to investigate experimental infection produced by parainfluenza-3 (SF-4) virus in laboratory animals and in various tissue culture
systems are in progress. Parainfluenza-3 virus multiplies readily in the
amniotic sac of 13- to 14-day-old chick embryos, before and after serial
passage, without losing its characteristic pathogenicity for primary cultures
of embryonic bovine-kidney cells. Infection was not maintained by serial
passage of egg or tissue culture virus in the allantoic cavity of younger
embryos. Attempts to demonstrate plaque formation by parainfluenza-3 virus
in primary monolayer cultures of embryonic bovine-kidney cells with agar
overlays were not successful.

#### H. Leptospirosis.

In 1961, at the Animal Disease Station at Beltsville, Leptospira pomona was grown in a medium in which the whole serum had been replaced by 1% albumin. Vitamin B<sub>12</sub> was required for growth. Ammonia may be a major source of nitrogen. No change in antigenic characteristics occurred.

Anaplasmosis infection did not protect cattle from infection with Leptospira pomona. Stress from leptospirosis triggered recurrences of anaplasmosis.

Leptospira pomona infection was fatal to chinchillas in as early as 6 days. Two animals inoculated with bovine kidney tissue from which Leptospira pomona was isolated by culture failed to become infected although they were susceptible to infection upon subsequent challenge.

No new infections have occurred in the Jeanerette Dairy herd where a long term vaccination study is in progress.

In 1962, at the National Animal Disease Laboratory, Ames, Iowa, Leptospira pomona and ll other major serotypes have been successfully cultured through an indefinite number of transfers in a medium whose major and most critical components are bovine albumin (Dubos oleic albumin complex DIFCO, Detroit, Michigan), Vitamin B-12, and ammonium chloride.

Maximum growth is achieved at a concentration of 1 percent albumin, although continuous subculture is possible at 0.25 percent albumin. Vitamin B-12 is the only vitamin which has been shown to be an absolute vitamin requirement in this specific medium. This need can best be demonstrated after subculture in the absence of added culture medium B-12. The amount of B-12 bound to the albumin has not been determined. No requirement for thiamin in this medium could be demonstrated. Consequently, thiamin has been deleted from the growth medium.

The ammonium ion source of choice is ammonium chloride. It is stimulatory at low levels, nontoxic at high levels, and cannot be replaced with monovalent cations. The deletion of ammonium ion still allows for continued growth in the presence of albumin.

Magnesium ion was found stimulatory to growth, but is not an absolute requirement for growth in the presence of the protein albumin. The stimulatory activity is not replaceable with divalent cations. The optimal level of sodium chloride in this medium is in the range of 0.27 - 0.37 percent.

The absolute need for trace metals is still in question and may remain so until deletion of the protein is achieved. The same is true of 1-cystine.

Minute inocula of the order of .25 percent as opposed to conventional 10 percent inocula will attain maximal cell crops during extended incubation periods in the current medium.

The growth supporting activity of the medium is not diminished by temperatures of 56° to 70° C. Complete destruction of growth supporting activity is seen at 82° to 84° C. after 30 minutes exposure.

This medium has been prepared in semi-solid form containing 0.2 percent regular agar and permits maintenance of stock cultures for prolonged periods of time.

The vaccination study in the Jeanerette dairy herd has continued. The fall 1961 bleeding was accomplished as planned but extensive personnel changes at Jeanerette interferred with the spring bleeding. To date no evidence of infection has been observed in either the vaccinated or nonvaccinated animals. The herd is gradually becoming seronegative through the coincidental removal of reactors in the routine culling process.

#### I. Epizootic Bovine Abortion.

In 1962, the University of California, Davis, under a cooperative agreement with the USDA, conducted investigations of epizootic bovine abortion (EBA). The studies under way include 1) attempts to ascertain whether the virus of EBA can induce abortion when administered either orally or nasally. These are believed to be the natural routes of infection. Should it be shown that the virus is capable of producing abortion when exposed to cattle through either or both of these avenues, it will remove any doubts that it might not be the sole cause of the condition. 2) attempts to determine whether cattle recovered from infection with the EBA virus are refractory to abortion when challenged with virulent virus. Such information is fundamental to any prevention program based on immunization. 3) Field trial studies to establish whether multiple injections of an inactivated EBA virus vaccine preparation will confer immunity to abortion. Contemplating that a viable agent will be needed to produce a satisfactory immunity, efforts are currently under way to attentuate the EBA virus by serial passage in tissue culture for possible use as an immunizing agent. 4) Epidemiological studies to ascertain whether the EBA virus is tick-transmitted and whether ticks are the reservoir of the virus in nature.

#### PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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# FOOT-AND-MOUTH DISEASE AND OTHER EXOTIC DISEASES OF CATTLE Animal Disease and Parasite Research Division, ARS

Problem. Responsibility for protection of the Nation's livestock industry against diseases, including those of foreign origin, was delegated to the USDA in 1884. Thereafter, contagious bovine pleuropneumonia eventually was eradicated from the United States, thus reopening European markets for practicable, scientifically justified barriers against introduction of such dangerous exotic diseases as foot-and-mouth disease and rinderpest. Plum Island Animal Disease Laboratory was established for scientific support of measures for protection against these and other foreign diseases of animals, following the direct threats of spread of foot-and-mouth disease from Mexico and Canada (1946-1954). Foot-and-mouth disease, which is capable of reducing over-all productivity by 25 percent in areas where it becomes established, persists in most major livestock producing countries, except Central and North America, Australia, and New Zealand. Rinderpest continues to be a serious disease problem in Africa and Asia; it is capable of killing 90 percent or more of the cattle that are exposed to it. Other diseases, such as contagious bovine pleuropneumonia, Rift Valley fever, and East Coast fever, continue to exact severe tolls in other parts of the world. Possibilities of entry of these diseases into the United States continue, despite all precautions, primarily because of the progressively increasing scope, speed, and extent of modern international transportation. The purposes of the Plum Island Laboratory are development of basic information applicable to protection of the Nation's livestock from foreign animal diseases; development and maintenance of competence in diagnosis of these diseases; and fundamental research on the biological, chemical, and physical properties of the infectious agents that may be useful in prevention, control, and eradication of these diseases.

#### USDA PROGRAM

The <u>Department</u> has a continuing long-term program involving veterinarians, biochemists, biophysicists, microbiologists, and pathologists, engaged in basic and applied research in this problem area. All of this research is being conducted on the following diseases at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York, except for supplemental field studies on vaccines in the Netherlands.

The <u>Federal</u> scientific effort devoted to research in this area, conducted solely at the Plum Island Animal Disease Laboratory, totals 33.3 professional man-years. This effort is divided among sub-headings as follows:

Pathological investigations of foot-and-mouth disease in cattle 1.0

Fluorescent antibody techniques 1.0

Diagnostic investigations 3.0

Susceptibility of cell lines 0.5

Production and maintenance of standardized reference stock of virus and homologous antisera 2.3

Carrier state in convalescent animals 0.5

Parasites in transmission of foot-and-mouth disease 0.5

Foot-and-mouth disease vaccines 4.0

Antigenic variations of foot-and-mouth disease viruses 1.0

Production of foot-and-mouth disease antibody in vitro 0.5

Immune response to various types and subtypes of foot-and-mouth disease virus 1.5

Quantity production of foot-and-mouth disease virus 2.0

Microcinematography of infected cells 0.5

Pure stable lines of culture cells 0.5

Purification of foot-and-mouth disease virus 2.0

Chemical and physical characterization of foot-and-mouth disease virus 1.0

Interaction between foot-and-mouth disease virus and host cells 1.0

Genetic biochemistry of foot-and-mouth disease virus 1.0

Effects of chemical and physical environments of foot-and-mouth disease virus 1.0

Preservation of foot-and-mouth disease virus 1.0

Rinderpest 2.5

Transmission of foot-and-mouth disease virus in semen 1.5

Survival of foot-and-mouth disease in meat and meat products 2.0

Susceptibility of wild species to foot-and-mouth disease 0.5

Adaptation of foot-and-mouth disease virus to poultry and embryonating chicken eggs 1.0

Public Law 480 funds equivalent to \$11,572.21 have been made available to the Turkish Ministry of Agriculture for a 2-year study of tissue culture of indigenous strains of foot-and-mouth disease virus and experimental field vaccination.

\$78,594 have been allotted to the Biological Institute, Sao Paulo, Brazil, for a 5-year study of tissue culture of indigenous strains of foot-and-mouth disease virus and experimental field vaccination.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

Experimentation with the virus of foot-and-mouth disease in the United States essentially is prohibited by law, except at the Plum Island Animal Disease Laboratory. Experimentations with the causative agents of other communicable, foreign, or exotic diseases of cattle in the United States is similarly prohibited generally by federal regulations. Consequently, the State Experiment Stations are not working with diseases in this category.

Insofar as is known, foot-and-mouth disease is the only one of the foreign diseases of cattle in which American industry has manifested notable interest. Although experimentation with foot-and-mouth disease is prohibited in the United States, except at Plum Island, at least two U. S. biological firms are known to have initiated vaccine production programs in South America, with plans for limited corollary research. It is estimated that no more than about 5 professional man-years are so engaged.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### A. Pathological investigations of foot-and-mouth disease in cattle.

In 1961, studies on the frequency of atypical (non-vesicular) lesions of foot-and-mouth disease in cattle were temporarily discontinued due to emphasis on other lines of cytological investigation. Spontaneous occurrence of so-called metastatic calcification syndrome among guinea pigs used for FMDV experimentation necessitated study of this disease on several occasions. Nephritis was found consistently in affected animals, but cytologic study and volumetric determinations of the parathyroid glands failed to show evidence of parathyroid hyperactivity secondary to renal damage. It was concluded that the calcification syndrome is of dietary, rather than renal origin, and that the scondary kidney lesions probably result from excretion of excess phosphates of dietary origin.

In  $\underline{1962}$  work on this project was limited to routine service work in histopathological diagnosis performed in connection with work in other projects. In addition, a large collection of kodachrome slides (256) and prints illustrating animals affected with exotic diseases was selected and furnished to ADE and the Economic Research Service.

# B. Fluorescent antibody techniques.

In 1961, two techniques were developed which may be used for demonstrating, localizing and identifying FMDV in tissues. In the indirect technique, the system consists of treating of FMD-infected tissue-culture cells with rabbit FMD antiserum, following which commercial fluorescein-conjugated sheep anti-rabbit globulin is added. The addition of commercial rhodamine bovine albumin to the FMD antisera and to the conjugate enhances the readability of positive reactions against normal rabbit serum controls by eliminating yellowish non-specific fluorescence. The direct method involves fluorescein-conjugated globulin from guinea pigs and bovine FMD antiserum (treated with liver powder and bovine albumin, to which rhodamine has been added to reduce non-specific fluorescence), with FMD-infected cell cultures. With these two techniques, the results indicate that high-passage tissue-culture virus may develop a broad antigenic spectrum. The results to date suggest these procedures may be useful in selection of strains of virus for vaccine production.

In 1962, the mass of antigen-antibody combination was found to be the most essential factor in obtaining fluorescent antibody reactions with foot-and-mouth disease virus (FMDV) that could be evaluated microscopically. A system including probable viral and cellular antigens combining with appropriate antibodies in convalescent bovine serum was developed for practical detection of cattle recovered from foot-and-mouth disease (FMD). The test developed is specific for FMD, but does not discriminate between virus types.

An indirect fluorescent antibody technique using rabbit-rinderpest serum proved satisfactory for demonstration of rinderpest antigen(s) in infected bovine kidney tissue cultures.

## C. Diagnostic investigations.

In 1961 preparedness for laboratory assistance in diagnosis of foot-and-mouth disease, and differentiation from vesicular stomatitis and vesicular exanthema, two clinically similar but distinct diseases, were considered of basic importance in the Plum Island program. Work is constantly under way in fundamental explorations aimed toward development of practical, accurate, and rapid means of identification of virus and specific antibodies.

In 1962 there was no report for work on this project.

#### D. Foot-and-mouth disease vaccines.

In 1961 the optimum conditions with regard to media, quantity of inoculum and time of harvest of virus grown in tissue cultures for large scale production of vaccine have been determined. Virus grown in bovine kidney tissue culture in bottles has produced a satisfactory vaccine for cattle at comparatively low cost and with potentially superior qualities. To date, virus grown in suspensions of trypsinized kidney cells has not been satisfactory for use in vaccine, apparently due to the low virus titers obtained by this method of propagation.

Continuation of studies on the safest methods of inactivation by formaldehyde has resulted in more rigid test procedures than previously were considered necessary. The testing of vaccine in 10 liter lots revealed new conditions which were not obvious when working with smaller experimental lots. In conjunction with these studies a method of testing for residual virus using tissue-culture methods is being explored, with indications that such a method may afford greater assurance of safety than testing in cattle only.

Correlation of measures of potency of vaccine in guinea pigs and cattle is continuing with indications that the response in vaccinated guinea pigs will permit estimation of the probable response in cattle to the same vaccine. A study of this type requires several simultaneous comparative trials in order to determine degree of correlation.

A mineral oil complex is being compared with standard aluminum hydroxide gel as an adjuvant in the vaccine. To date, data indicate the new adjuvant is as good as and possibly superior to the aluminum gel. When the mineral oil complex was used, the antibody response was 100-fold higher in some animals.

Vaccines produced for use in cattle are being tested concurrently in swine. There appears to be a fundamental difference between the two species in vaccine response; swine generally have responded poorly to vaccine produced from virus propagated in tissue cultures. There are some indications, however, that the response in swine may be a function of the adjuvant used in the vaccine and the route of injection of the product.

Comprehensive studies under way in Europe on the duration and extent of immunity in cattle following vaccination with FMD vaccine require long periods of time and many animals. Such investigations are difficult if not infeasible under laboratory conditions. An ADP representative is stationed in Amsterdam, as Chief of the Division's European Mission for Research on Animal Diseases, and such studies are in progress in the Netherlands in cooperation with Dutch researchers. Tests involving 2 herds of Friesian cattle have been in progress for approximately 2 years. Serologic studies in the 2 herds have been continued. Results obtained during the past year have warranted extension of the program to include additional herds. This will afford more significant numbers of animals for challenge with virulent FMD virus and permit correlation of resistance to infection with the index of immunity as measured by virusneutralization tests of serums.

To obtain sufficient animals for challenge, 10 additional herds have been included in the study. The total number of animals under observation is approximately 400. It is estimated that of this number about 40 annually will become available for challenge. The substantial increase in the number of samples to be tested as a result of this change has justified comparative studies between the virus-neutralization test, depending upon complement fixation, and another test, depending upon changes in pH.

Serums of animals which have experienced two or more annual vaccinations continue to show a high level of antibodies. Two animals showing high levels of antibodies were resistant to challenge with virulent virus, as indicated by absence of generalized infection. Results to date promise eventual development of sound techniques for evaluation of vaccines prepared by various methods, and scientific application of vaccines in the field.

In 1962 cattle vaccinated with inactivated FMD vaccine containing emulsified oil as an adjuvant developed a significantly better protector than cattle receiving a similar product containing aluminum-hydroxide gel as an adjuvant. Study of virus-neutralizing antibodies revealed high antibody levels at 9 months in cattle vaccinated with the emulsified oil preparation, whereas, cattle vaccinated with vaccine containing aluminum-hydroxide gel had low antibody levels at four months. Challenge of these cattle by exposure to live virus showed that the cattle which received the oil emulsion vaccine were better protected.

#### E. Antigenic variations of foot-and-mouth disease viruses.

In 1961 the susceptibility of mother mice to FMDV began to decline about 3 weeks post-partum until the 5th week when they became almost completely resistant. Substantial reduction of the number of suckling mice 2 days before inoculation of mother mice appears to increase their resistance. Cells from peritoneal exudate of susceptible mother mice exposed in vitro to FMDV have shown no dimunition of phagocytic activity (for yeast) nor was virus absorbed or propagated in these cells. Increasing the number of white cells in the peritonea of mother mice before infection with FMDV did not appear to alter their susceptibility.

In 1962 factors were investigated that might affect the susceptibility of mother mice to FEDV. Virus from tongue epithelium of infected steers and virus from the first few passages in tissue culture produced a low mortality in mother mice, but after 6-8 serial passages in tissue culture the virus killed 40-70% of the mothers. Beginning approximately three weeks post partum, mother mice, which were resistant to the virus before becoming pregnant, gradually became resistant again. The ability of mother mice to respond immunologically is impaired as demonstrated by a hypersensitivity response to bovine serum. The response is lower in both mother mice sensitized seven days post partum and in mother mice sensitized before being bred.

Basic studies under this project have shown that bovine serum antibody may be assayed in the complement-fixation test using guinea pig vesicular fluid as an antigen. Study of sheep serum has shown it to have good complement-fixing antibody and apparently none of the qualities of bovine serum that make CF tests difficult. Comparison of antigens from different sources such as tissue culture and guinea pig vesicular fluid have led to studies to establish antigen standards. Such standards are essential for the serological testing of vaccine antigenicity.

# F. Immune response to various types and subtypes of foot-and-mouth disease virus.

In 1961 serological studies were continued on a group of cattle three years after infection and a detectable level of circulating antibody has persisted. Studies have included examination of the animals for latent virus infection to determine whether the persisting antibody level may be due to intracellular virus which has continued to stimulate antibody production. Another approach has been inoculation of pituitary hormones to produce physiological stress which might cause animals to shed latent virus. Although the results to date have been negative, the existence of residual virus in these animals has not been disproved.

In 1962, detailed studies of transfer of immunity from vaccinated cows to their calves have shown this to be through the colostrum only. It has been shown that a calf is born free of serum gamma globulin and only receives it through maternal colostrum. The duration of this passive immunity is dependent on the antibody content of the colostrum received by the calf when it first nurses.

#### G. Quantity production of foot-and-mouth disease virus.

In 1961 work was initiated on devising methods for rapid and economical production of large quantities of FMDV in surviving kidney-cell suspensions. Steps in the operation being studied included (1) areas of renal tissue providing best viral growth, (2) methods for mincing large quantities of tissue, (3) trypsinizing procedures, (4) types of culture vessels, (5) media, (6) growth conditions, including cell concentration, depth of culture, agitation and alteration, and (7) fragmentation of infected cells for virus release.

In preliminary work, cultures of FMDV, type A, strain 119, have been attained with infectivity titers somewhat higher than the titer considered satisfactory for vaccine production in foreign countries (10<sup>6</sup> infectious units per ml.). Methods of production of large quantities of FMDV, type A, strain 119, in bovine kidney cells cultured on glass have been improved by providing better conditions for the cells for viral growth. This has resulted in a 50 percent increase in susceptibility to infection and in virus yield.

In 1962 methods were developed for rapid and economical production of large quantities of foot-and-mouth disease virus (FMDV) in stationary suspensions of trypsin-dispersed bovine kidney cells in a simple medium. Yields of between 107 and 100 plaque-forming units (PFU) per milliliter were obtained from serum-free cultures containing approximately a million and a half viable trypsin-dispersed cells per milliliter. Yields of up to 106.9 PFU were obtained from simple cultures of finely minced calf kidney tissue provided cell debris was removed from the minced particles by trypsinization.

### H. Microcinematography of infected cells.

In 1961 tissue cultures infected with type A, strain 119, FMDV were photographed. The cytoplasm of infected cells contracts around the nucleus leaving small branching streamers of protoplasm attached to the glass resulting in a bush-like or tree-like alteration of the cytoplasm. The term "arborization of the cytoplasm" was applied to this stage of the degenerative change.

Normal cells undergoing mitosis also contract but their cytoplasm fails to undergo arborization. After the degenerating cells have undergone arborization, they undergo a form of activity described as "boiling", and then detach from the glass. Critical evaluation of preliminary films indicated the need for substantial modifications of the equipment to provide longer exposure time at higher magnification. This was accomplished, and considerable experimentation with different methods of film processing was made to provide high-quality film.

In 1962 improvements in microcinematographic technique have resulted in production of films satisfactory for demonstration and possible distribution. Microcinematographic studies have been made on growth of normal primary calf-kidney cultures and cultures infected with foot-and-mouth disease (FMDV) and rinderpest virus. Films prepared have been shown at scientific meetings. The phenomenon of cell survival was noticed and confirmed in the course of the above studies. A small portion of the cell population in primary calf-kidney cultures resists the action of FMDV. The cells not destroyed by the virus continue to multiply. Virus in low titer persists in the surviving cultures but a small-plaque moiety becomes predominant. Studies on changes developing in virus populations persisting in surviving cultures are being made.

# I. Pure stable lines of culture cells.

In 1962, a manuscript was prepared with abstract as follows: Continuous culture of lamb testis cells on glass and in agitated fluid suspension cultures has been achieved several times. No obvious cell alterations occurred during more than 40 serial passages. The culture system appeared to be well suited for production and assay of foot-and-mouth disease and some other animal viruses. Several additional established cell lines from outside sources were tested for susceptibility to foot-and-mouth disease virus with negative results.

# J. Purification of foot-and-mouth disease virus.

In 1961, a rapid method of centrifugation for purifying FMDV was developed which combined isodensity separation immediately below a moving zone separation. A preformed density gradient of cesium chloride enabled the virus to band into a narrow zone within 4 hours at 37,000 rpm in a swinging bucket tube. The viral light-scattering zone when removed at a concentration of 8-fold had 47± 16% of the original infectivity and contained virus particles as revealed by electron microscopy. The cesium chloride isodensity value of FMDV of 1.43 ± 0.01 g/ml was significantly higher than that of protein contaminants.

Combinations of basic types of centrifugation include sedimentation of FMDV through an interface formed by an aqueous phase and an immiscible organic fluid. Separation of virus from contaminants appears to depend upon hydrated particle densities and specific denaturation by organic fluids, in contrast to the dependence upon anhydrous particle densities in cesium chloride gradients.

Certain viruses, particularly bacteriophages, are stable only in the presence of magnesium ions. This ion, however, was found to have no stabilizing effect and possibly even detrimental to FMDV infectivity. This observation prompted use of the chelating agent, sodium ethylene diamine tretraacetate (EDTA) to remove bivalent cations from the highly purified virus described above. In 1% EDTA infectivity was essentially constant over a 55-day period at -60C; longer periods have not been investigated. EDTA can be readily removed by dialysis.

In 1962, a new ultracentrifugation technique termed organic interface centrifugation was perfected. It combines in one run, moving boundary centrifugation, isodensity purification and organic extraction. Foot-and-mouth disease virus is being purified and concentrated from infectious tissue culture fluids by procedures incorporating alcohol precipitation, organic extractions, cesium chloride density gradient centrifugations and organic-interface centrifugations. The average weight of virus obtained from 10 liters of fluid was 100 micrograms based on electron microscope counts. This work will be continued until foot-and-mouth disease virus of at least 95% purity is obtained. Progress has been limited by the lack of a virus production unit at Plum Island.

# K. Chemical and physical characterization of foot-and-mouth disease virus.

In 1961, foot-and-mouth disease virus (FMDV), type A, from infected guinea-pig foot-pad vesicular fluid was examined for fine structure in both phosphotungstic acid and uranium-shadowed preparations on carbon coated grids. Phosphotungstic acid penetration was less than that reported with many other viruses,

hence shadowed specimens were also used extensively to study the structure. Regular polyhedral models of 12, 20, 32, and 42 subunits were constructed and analyzed for distinguishing features exhibited by both virus and model. Mixtures of FMDV and bacteriophage which have approximately the same diameters, revealed that FMDV had smaller and more numerous subunits. The icosahedral model with 42 subunits was favored over the modified dodecahedral model of 32 subunits for FMDV, although there was no unequivocal evidence that these represented the ultimate structure of FMDV. A carbon shadowing device was modified to facilitate studies of ultra-structure. The improvement consisted of a tungsten metal spring for advancing the pointed carbon rod toward the cavity in the blunt rod.

In 1962, foot-and-mouth disease virus was compared in the electron microscope with a bacteriophage and Turnip yellow mosaic virus (32 subunits). The number of subunits in foot-and-mouth disease virus exceeded that of the bacteriophage, but the exact number could not be determined. Complications in ultracentrifugation calculations introduced by the use of variously shaped cells were simplified by using higher mathematical functions in tabular form. Tabulation of log values required for sedimentation coefficients permits rapid calculation of the minimum s-rate for sedimentation for a known volume.

# L. Interaction between foot-and-mouth disease virus and host cells.

In 1961, cultured bovine cells, partially depleted of endogenous metabolites and maintained on a medium with glucose as the sole organic material, were examined for acid production by known chromatographic procedures. The kinds of acids detected and their rates of production were the same for both normal and infected cells. Lactic acid constituted about 90% of the total acidity with acetic acid being the only other acid identified during the 12-hour post-starvation period studied. Concentration of acetic acid was highest during the initial recovery period of the starved cells. Cells infected with FMDV yielded comparable results although acetate production appeared to persist for longer periods of time. No acids of the tri-carboxylic acid cycle were detected in either infected or uninfected cells.

Cells partially depleted of endogenous nutrients utilized pyruvate at a rate 2.5 to 4 times greater than glucose. The pathways of pyruvate metabolism were not clearly defined. Pyruvate was not appreciably metabolized oxidatively since oxygen uptake could account for only 6-2% of the pyruvate consumed and malonate only slightly decreased its utilization. Substrate pyruvate did not form lactate and was used to a greater extent as glucose concentration decreased. Glucose utilization was independent of pyruvate concentration. Virus infection did not change the rate of pyruvate utilization in contrast to the increase found in glucose uptake. Lactalbumin hydrolyzate did not yield lactic acid of itself but did stimulate glucose uptake and corresponding lactate formation. Acetate was not oxidized nor was lactate produced. Cells uninfected with FMDV maintained the same pattern of glucose metabolism as did uninfected cells.

Thin sectioning for electron microscopy has been started with the examination of sedimented cells from normal and FMDV-infected bovine kidney cultures as well as with tissues infected with rinderpest virus. Training of a technician in routines of fixation, embedding, sectioning and examination of the thin sections has been started. Studies are planned and in progress for following the synthesis of FMDV and of rinderpest virus in cultures.

In 1962 further chemical investigations of organic acids synthesized by bovine kidney culture cells grown in media with and without serum showed lactic acid to be the major component, accounting for 86 to 90% of the total, while acetic and pyruvic acids accounted for 7 to 9% and 3 to 6% respectively. Foot-and-mouth disease virus-infected cultures, independent of medium, produced more of these acids than uninfected cultures. Studies with cultures partially depleted of endogenous nutrients and exposed to serum-free medium indicated that acetic and pyruvic acids may be derived from an endogenous substrate other than glucose. The contribution of lactic acid to total acidity increased with increasing glucose concentrations.

The above chemical and other metabolic studies of glucose metabolism in bovine kidney culture cells were confirmed by radioisotope tracer studies. Extracellular acids account for nearly all of the Cl4 with over 90% residing in lactic acid. Intracellular isotopic activity, 1 to 3% of the total, was distributed among the cold trichloracetic acid soluble pool, hot trichloracetic acid, lipid and protein fractions.

Purine and pyrimidine analogues were studied for possible inhibition of foot-and-mouth disease virus synthesis in bovine kidney culture cells. Eight analogues at 250 ug/ml did not alter cellular metabolism measured in terms of oxygen consumption and glucose utilization. When tested for inhibition of virus synthesis 8-azaguanine, dithouracil and 5 bromouracil sometimes decreased the amount of recoverable virus. The latter compound gave the most consistent decreases, and the inhibition was not prevented by adding natural pyrimidines, uracil and thymine. Interpretations are not clear-cut because controls on virus stability in vitro with the analogues showed that 8-azaguanine and dithouracil had no effect, while 5 bromouracil caused significant losses in activity.

#### M. Genetic biochemistry of foot-and-mouth disease virus.

In 1961 the presumption that FMDV splits off infectious ribonucleic acid (RNA) when heated has been substantiated. The tedious job of removing all ribonuclease (RNase) from virus preparations was accomplished. The resulting RNase-free virus yielded infectious RNA when heated at 61 and 85 C. Bentonite has been found to eliminate the last traces of RNase from phenol-derived RNA. Such RNA may be stored for at least 3 months at -60C with undiminished infectivity. Heretofore, phenol-derived RNA retained full infectivity only when stored in liquid nitrogen.

The precision of the plaque assay for FMDV-RNA was determined. Randomizing older data for 100 replicate platings of RNA in two ways, i.e., into groups of 5 and 10, gave respective ranges with one standard deviation of 27.2 ± 21% and 54.3 ± 16%. New data for 50 replicate platings gave a range of 17.5 ± 27%. Thus, plaque counts ranging between 17 to 54 are known to a precision of 27% or better 65% of the time. The plating efficiency of FNDV-RNA is increased nearly 10-fold by inclusion of slightly soluble substances in the inoculum. The best substance found thus far is Attasorb.

Work is continuing on an apparent RNase inhibitor in calf-kidney cell cultures. The RNase-like activity of cell extracts inactivating FMDV-RNA is potentiated markedly by heating, pH changes and by the protein-protein dissociating agent, p-chloromercuribenzoate (pCMB). The optimal temperature range for thermal potentiation is 55 to 65 C with activity increasing as much as 1000-fold. If sodium dodecylsulfate (SDS) is present prior to heating, the RNAse-like activity of cell extracts is potentiated to a lesser degree. This is in accord with the finding that virus heated in the presence of SDS yields infectious RNA, with the hot SDS inactivating environmental RNase. Transient acidification or alkalization of cell extracts increases their RNase-like activity by 10-fold or greater. pCMB also effectively increased the ability of cell extracts to destroy RNA infectivity. Dissociated inhibitor and RNase do not recombine readily.

In 1962 kinetic curves for the thermal inactivation of FMDV-RNA, freed with bentonite of the last traces of ribonuclease, were determined at 7 temperatures over a 3 hour period. Such RNA is not pure and contains much cellular RNA and DNA. The rates were first order with 1.5-hour survivals of 0.095, 0.85, 0.45, 0.25, 0.08 and 0.006 at 1°, 26°, 37°, 45°, 55° and 61°C, respectively. Survival was only 0.001 after 10 minutes heating at 85°C. The primary structure, secondary structure and reactivity of infectious FMDV-RNA obtained from pure FMDV is under investigation.

# N. Effects of chemical and physical environments of foot-and-mouth disease virus.

In 1961 a rate study of the inactivation of the virus with AEI (acetylethylenimine), BPL (betapropiolactone), and ETO (ethylene oxide), was performed at 23 °C. To 250 ml. volumes of A-119 virus in the 89th tissue-culture passage 0.05% AEI, 0.05% BPL, or 0.5% ETO was added. Samples were taken at 2-hour intervals in the critical period of inactivation. In innocuity tests in pairs of cattle when 2 ml. of each sample was given IDL, the results were as follows: AEI in a concentration of 0.05% inactivated the virus in 22 to 24 hours, BPL in a concentration of 0.05% inactivated the virus in 12 hours, and ETO in a concentration of 0.05% inactivated the virus in 16 hours. The cattle which showed no signs of FMD were challenged after 14 days with 106.2 bovine ID50 by the intramuscular route. In the cattle that had been injected with virus inactivated by AEI in a 24-hour exposure, no primary or secondary lesions of FMDV were found in the 14-day observation period. In the cattle inoculated with virus in the presence of BPL for 12 hours, both tongue and foot lesions developed during the post-challenge observation period. Injection

of virus in the presence of ETO for 16 hours permitted development of tongue and foot lesions in the challenged cattle. It appears from these studies that AEI is superior to BPL and ETO as an inactivant. Ancillary tests such as virus-neutralizing capacity of the serum of chickens injected with 1 ml. of the same preparations as given to the steers, and the virus-neutralizing capacity of adult mouse serums after injections with the inactive viral preparations confirm the foregoing assumption. The following titers in PFU/ml have been obtained with A, O, C, SAT-1, SAT-2, SAT-3, and Asia-1, respectively, after 89, 22, 22, 8, 8, 8, and 10 tissue-culture passages: 8.0, 7.8, 7.6, 8.2, 7.7, 6.3, and 7.3, in that order. The titers in mice as LD approximate the tissue culture PFU/ml.

In 1962, studies on chemical inactivation of foot-and-mouth disease virus (FEDV), 0.05% acetylethyleneimine (AEI), at a temperature of 23°C for 24 hours, inactivated FMDV propagated in tissue cultures. The effects of beta-propiolactone (BPL) were also studied and were found to be less reliable than AEI. FMDV preparations inactivated with AEI retain the greater part of their antigenicity while BPL is more severe on viral antigenicity as tested in cattle, chickens, and mice. Cattle used in the infectivity studies were injected in the tongue and were later shown to be immune when challenged by the intramuscular route with concentrations of virus as low as 10,000 bovine ID<sub>50</sub>.

Twenty nine cationic and anionic surface active agents were tested for virucidal effect against Type O-Mll FMDV. Only one chemical, methyl ethyl isoquinilinium chloride, was capable of destroying 3-4 logs of viral infectivity in 2 hours at 28 C, whereas others only inactivated between 1-2 logs of virus under the same conditions.

# O. Preservation of foot-and-mouth disease virus.

In 1961 tissue culture-propagated virus and virus in bovine tongue-tissue suspensions were used for storage in three replicate experiments. To lots of tissue cultured A-119 virus in the 88th passage 5% gelatin, 5% sucrose, or 0.2% cysteine were added, respectively. Antibiotics in concentrations of 1000 units of penicillin and 1 mg. of streptomycin were added per ml. Samples of each preparation were stored at 37, 23, 4 and -50 C in flamesealed ampoules. Lots of 10% suspensions of tongue tissue from cattle infected with A-119 virus in the 9th and 10th bovine passages were supported with 2% gelatin, 5% sucrose, 50% normal bovine serum, L.C. fluid (tissue culture medium), or tryptose phosphate broth. Antibiotics as described above, were also added. Samples of the preparations in flame-sealed ampoules were stored at 37, 23, 4 and -50 C. Infectivity studies of the various preparations were conducted in suckling mice. At the end of a year, it was evident that virus of tissue-culture origin with or without additives, was more stable at 37, 23 and 4 C than was virus in tissue suspension. Both forms of the virus retained the approximate original titers when held at -50 C for a year. Tissue-culture virus survived at least 7 days at 37 C, for 8 weeks at 23 C, and 12 months at 4 C. Virus in tissue suspension survived for 2 days at 37 C, 2 weeks at 23 C and 6 months at 4 C. The additives gave little if any supporting effect except in tissue suspension supported by 50% bovine serum at 4 C.

Cysteine had an apparently harmful effect. The freeze-drying was performed on an Edward's freeze-drying machine. It was established previously that a condenser temperature of -50 C must be maintained in the primary phase of drying with a vacuum of 150 mm or less of mercury throughout a period of 20 hours, followed by a vacuum of 80 mm for the secondary phase of drying over P<sub>2</sub>O<sub>5</sub> for 4 hour period at 23 C. Virus from tissue cultures and in tissue suspensions was dried and stored in 10 ml ampoules in 4 ml. amounts. Additives in the following concentrations were used singly or in combination with tissue culture virus: 5% sucrose, 2% gelatin, 2% dextrin, 2% sucrose, 5% skim milk powder, 1% glutamate, and 5% normal bovine serum. Tryptose phosphate, L. C. fluid, 5% normal bovine serum, 5% glutamate, and 5% skim milk powder were used singly or in combination with virus in tissue suspension. All vials were flamesealed under vacuum after drying. Titration of samples for infectivity was conducted in suckling mice. In the presence of perfect mechanical conditions it has been possible to dry the virus with only 0.5 to 1 log loss in infectivity. Tissue-culture virus without supportive preparations dropped in titer from 0.5 to 1 log of virus following storage for one year. However, for best storage 5% skim milk powder or a combination of 5% sucrose, 5% bovine serum, and 1% glutamate concentration should be added. Virus in tissue suspensions stored well for a period of a year with tryptose phosphate or L.C. fluid as diluents. Data is not complete for all lots of freeze-dried virus in storage for a year.

In 1962, type A-119 foot-and-mouth disease virus (FMDV) was propagated in tissue cultures and in the dermis of cattle tongues. Various proteins, carbohydrates, or salts of amino acids were added to both types of viral preparations. Samples of these materials in 4-ml. amounts in flame-sealed ampoules were held at 37, 4, 23, and -50 C for periods up to a year. Virus produced in tissue cultures survived at least 7 days at 37 C, for 8 weeks at 23 C, and 12 months at 4 C. Virus in tissue suspensions survived 2 days at 37 C, for 2 weeks at 23 C, and for 8 months at 4 C. None of the additives significantly increased the storage survival. Virus from both sources, with or without additives, was stable for a year at -50 C. All infectivity tests were conducted in mice.

Similar preparations of Type A-119 FMDV were prepared with various additives such as proteins, carbohydrates, or salts of amino acids. These preparations were freeze-dried in 4-ml. amounts in ampoules in the Edward's freeze-dryer and stored for a year at 4 C. In most instances the over-all loss of virus in processing and storage did not exceed a log in infectivity. There was no apparent difference in storage stability between the two sources of virus and the additives apparently did not appear to influence the storage qualities.

## P. Rinderpest.

In <u>1961</u> some dogs inoculated with rinderpest virus developed antibodies to rinderpest and resisted challenge with distemper virus. Cattle inoculated with measles and distemper viruses failed to develop antibodies against rinderpest virus. Inoculation of monkeys with distemper and rinderpest viruses and subsequent challenge with measles virus will complete the study.

Three strains of rinderpest virus passaged in tissue cultures have been reduced in virulence for cattle. All strains tested have been shown to share common antigenic factors. Some such strains may be useful as immunizing agents.

Although some progress has been made in electron microscopy studies, the virus particle of rinderpest has not yet been identified. It has been found that the virus is unstable in butanol and other lipid solvents. The virus was readily precipitated by centrifugation, but after being pelleted it could not be fully resuspended. Thus, purification procedures employing lipid solvents and pelleting have not been applicable. This ruled out, for the present at least, the possibility of using lymph nodes as a source of virus because of their relatively high content of impurities. Progress has been made, however, in concentrating and purifying tissue-culture virus. When the virus was found to withstand high salt concentration, it was possible to zone it by centrifugation in cesium chloride density gradients with nearly complete recovery of infectivity, 100-fold concentration, and the elimination of much debris. Such material after dialysis to remove salt, revealed a few virus-like particles, approximately 100 millimicrons in diameter, but they were too few to permit correlation with infectivity. The virus was concentrated about 100-fold by dialysis against polyethylene glycol, and its infectivity was unaffected by polyethylene glycol.

In 1962 relationships of the viruses of human measles, canine distemper and rinderpest were studied. Cattle injected with measles or distemper virus were not protected against challenge with rinderpest virus, although distemper antibodies were found in cattle injected with that virus. Each of the viruses elicited antibody response in an alien host. Puppies injected with either measles or rinderpest virus were protected against distemper.

Work was continued on the attenuation of the Kabete strain of rinderpest virus in tissue cultures. The modified virus retained sufficient antigenicity to immunize cattle, and in limited studies, it produced only limited clinical response. This strain of virus was inactivated at pH 2 and 12; but at pH 3, the virus remained viable for 5 minutes, and at pH 11, for 20 minutes. Virus-cell relationships were studied by electron microscopy. From this work, it is apparent that rinderpest virus develops in the mitochondria of infected bovine kidney cells in cultures.

Cross-immunity studies were made with one strain of bovine virus diarrhea and rinderpest. Calves recovering from experimental reinfection with virus diarrhea were susceptible to challenge with rinderpest virus, and serum from animals with virus diarrhea did not cross-react with rinderpest virus. Calves experimentally immunized against rinderpest, did not develop antibodies to virus diarrhea virus, and such animals were susceptible to challenge with virus diarrhea virus. These results indicate lack of serological or immunological relationship between the two viruses.

## Q. Transmission of foot-and-mouth disease virus in semen.

In 1961 guinea pigs, bulls and heifers were used in preliminary studies. Guinea pigs and steers were used for infectivity studies. Type A, strain GB, FMDV was used in the studies in guinea pigs and types A, strain 119, and O-M11 were used in cattle.

Virus was found in the urine, testicles, vas deferens and epididymus of male guinea pigs 48 hours after inoculation via the metatarsal pads, but was not found in the seminal vesicles. The titer of virus in the vas deferens was higher than that observed in the other tissues and fluids. Virus was detected in the testicles 24, 48 and 72 hours after inoculation. Female guinea pigs showed lesions of FMD 120 hours after instillation of virus in the vagina or rubbing on the vulva. Other female guinea pigs failed to develop FMD when virus, diluted 1:10 with milk or egg yolk semen extenders, was instilled into the vagina. Control animals inoculated with diluted virus intradermally in the foot pads developed lesions within 24 hours. In limited trials, urine from infected male guinea pigs produced lesions when inoculated into the foot pads, but failed to produce FMD infection when instilled in the vagina of susceptible guinea pigs.

Two bulls were inoculated on the tongue with Type A, strain 119, FMDV and slaughtered 24 hours later, when signs and lesions of infection were evident in only one of the two animals; however, both developed viremia (titers of  $10^{4.8}$  and  $10^{4.5}$  ID<sub>50/ml</sub>, respectively). The testicles, epididymus and lining of the bladder from both animals contained infective virus. Virus was not found in the seminal vesicles or lining of the urethra from either bull, however, infective virus was demonstrated in the vas deferens from the bull which developed clinical signs of FMD prior to slaughter.

Heifers in oestrus and not in oestrus were used. The virus was prepared from infected bovine tongue tissue in various suspending fluids. Approximately 3.0 ml. of virus suspension was instilled in the vagina at a depth of about 12 inches, using precautions to prevent contamination of the vulva and surrounding area. Of two heifers receiving a bovine semen-virus preparation, one, which had been in oestrus 4 days prior to vaginal exposure, developed FMD within 4 days. The heifer not in oestrus did not develop clinical evidence of FMD and was susceptible to subsequent inoculation. Four heifers received the virus diluted with egg-yolk extender. One of three heifers in oestrus at time of exposure developed clinical FMD. The other two heifers remained free of evidence of infection and were susceptible to a subsequent inoculation. One heifer not in oestrus at the time of vaginal exposure developed signs and lesions of FMD 5 days later. Two additional heifers not in oestrus developed FMD within 4 days after vaginal exposure with 10 per cent suspension of Type O, strain M-11, virus diluted equally with bovine semen.

In 1962 semen samples were collected at various intervals after inoculation from 8 FMD-infected bulls by use of an electroejaculator. Urine and blood samples were also taken. Infectivity studies were conducted in mice and steers. FMDV (A-119 or 0-M11) was found in the semen and urine of bulls as early as

12 hours and in blood as early as 6 hours after inoculation. During convalescence, virus was found in semen and urine for as long as 7 days and in the blood for as long as 4-3/4 days after inoculation. The titer of the virus in the semen usually was higher than in urine and sometimes higher than in the blood. The pH of the urine usually shifted from alkaline to acid about 2 days after inoculation and then returned to alkaline in about a week as the bulls recovered from acute infection. The pH of semen varied, but remained nearly neutral throughout the course of the disease.

## R. Survival of foot-and-mouth disease in meat and meat products.

In <u>1961</u> studies previously reported demonstrated that FMDV may be found in lymph nodes, hemal nodes, bone marrow and large blood clots in carcasses of infected animals ripened at 4 C for 72 hours. The studies were extended to determine if virus also could be detected in bone marrow and lymph nodes of animals slaughtered at times after the initial stages of infection.

Virus was demonstrated in fresh rib bone marrow of steers for as long as 3 days after inoculation with FMDV A-119. Virus was not found in bone marrow of steers slaughtered 4, 5 and 9 days after inoculation with FMDV A-119, nor was virus found in bone marrow of a steer 7 days after inoculation with FMDV type SAT-3.

All 7 types of FMDV were used to determine the length of time after inoculation that the various viruses could be detected in lymph nodes. The number of days after inoculation that the various types and strains of infectious FMDV survived in lymph nodes of infected steers was as follows: 9 days for O-M11, C-149 and Asia 1; 11 days for A-119 and SAT-3, and 13 days for SAT-1 and SAT-2. However, on the 13th day post-inoculation, even though the test steers did not become clinically ill from the material inoculated from A-119 and C-149 infected donors, some of the test steers were sufficiently immunized by the material to resist subsequent challenge and had significant virusneutralization indices, 3.7 and 5.5, respectively.

The titer of FLDV in the head and body lymph nodes of infected donor steers was compared on various days after inoculation, using 3 strains of FMDV (SAT-1, O-Mll and A-119). As might be expected, since the steers were infected by tongue inoculation, the titer of the virus was usually slightly higher in the head lymph nodes (mandibular primarily) than in the body nodes. For the first 3 days after inoculation the virus titers in the lymph nodes varied from 1.3 to 4.5, with an average of 3.2 (bovine ID<sub>50</sub> or mouse LD<sub>50</sub> per ml. based on 15 titrations). At the 7th, 8th and 9th days after inoculation, the titers varied from 1.3 to 3.0, with an average of 2.4 for 5 observations. No virus was demonstrated in 11 other samples tested. Virus titers as high as 5.6 have been obtained from the blood of steers on the third day after inoculation with FNDV A-119, and 7.8 or higher from infected tongue tissue, while the titer in lymph nodes has not exceeded 4.5

In 1962 ground meat, composed of lymph nodes and muscle tissue, in a ratio of 1:10, was prepared from FED-infected steer carcasses stored at 4 C for 72 hours. The ground meat was stored at 4 C, unsalted and salted (4% NaCl). When fluids were expressed from unsalted meat, FMDV was found as long as 11 days, but could not be detected in salted meat stored for more than 4 hours. However, when the lymph-node fragments were sorted from the ground meat and tested, virus was found in salted meat for as long as 17 days.

The titer of FMDV in lymph nodes declines during storage; 34 samples gave an average titer of 10<sup>3.0</sup> (fresh) and about 10<sup>1.6</sup> after 9-10 days storage. FMDV in lymph nodes taken during the pre-clinical stage of the disease apparently does not survive as long during storage as virus in lymph nodes taken after the disease is well established.

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# PARASITES AND PARASITIC DISEASES OF CATTLE Animal Disease and Parasite Research Division, ARS

Problem. The cost of parasitic diseases to the cattle industry of the United States is estimated to be in excess of \$400 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy cattle, insure adequate supplies of parasite-free beef for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a more prosperous agriculture and the national economy.

#### USDA PROGRAM

The <u>Department</u> has a continuous long-term program involving biochemists, microbiologists, parasitologists, pathologists and veterinarians engaged in both basic and applied studies directed to the development of measures for the solution to the high and extremely costly incidence of parasitism in cattle. Research is being conducted on parasitic diseases at the designated locations.

The <u>Federal</u> scientific effort devoted to research in this area totals 17.7 professional man-years. This effort is divided among subheadings as follows:

Ecological Factors Influencing Nematode Development 1.1 at the Regional Animal Parasite Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Effects of Mixed Helminth Infections 2.0 at the Regional Animal Parasite Laboratory, Auburn, Alabama.

Acquisition of Parasites from Pastures 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Effect of Pasture Mixtures and Pasture Management on Control of Internal Parasites 1.5 at the Regional Animal Parasite Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Winter Coccidiosis (Bloody Scours) 1.0 at the Regional Animal Disease and Parasite Laboratory, Logan, Utah, and under a cooperative agreement with the Montana Agricultural Experiment Station, Bozeman.

Influence of Diet and Nutrition of Cattle on Roundworm Parasitism 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Artificial Propagation of Protozoan Parasites 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Host-Parasite Relationships of Coccidia 1.0 at the Regional Animal Parasite Laboratory, Auburn, Alabama.

Ecology and Immunology of Lungworms 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle O.1 at the University of California, Davis under a cooperative agreement with the USDA.

Investigations of Trichomonad Parasites 1.0 at the Regional Animal Disease and Parasite Laboratory, Logan, Utah, and under a cooperative agreement with the Utah Agricultural Experiment Station, Logan.

Host-Parasite Relationship of Intestinal Worms Cooperia spp. 2.0 at the Regional Animal Parasite Laboratory, Auburn, Alabama.

Anaplasmosis 4.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and through memoranda of understanding and other arrangements in cooperation with State Experiment Stations in California, Illinois, Louisiana and Nevada, the State Veterinarian of Tennessee, the USDA Entomology Research Station, Kerrville, Texas, the Delta Branch Experiment Station, Stoneville, Mississippi, and a large cattle ranch in Virginia and in Wyoming.

Investigations on Anaplasmosis, Piroplasmosis and Babesiellosis of Cattle, are under way through a PL 480 Grant, at the School of Veterinary Faculty, Montevideo, Uruguay.

Investigations on the Pathogenesis of Lesions Produced by the Leech, <a href="Limmatus nilotica">Limmatus nilotica</a> are under way at the Hadassah-Hebrew University Medical School, Israel, under a PL 480 grant.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 21.7 professional man-years divided among subheadings as follows: Ecological factors influencing nematode development 2.4; mixed helminth infections 4.4; acquisition of parasites from pastures 3.9; winter coccidiosis (bloody scours) 1.0; influence of diet and nutrition of cattle on roundworm parasitism 0.9; clinical and physiological aspects of roundworm parasitism in cattle 1.1; anaplasmosis 6.9. Nine southern and four western States and

the USDA cooperate in regional research (S-21, Gastrointestinal Parasites of Ruminants, and W-35, Nematode Parasites of Ruminants) to determine the Ecological Factors Influencing Nematode Development. The two regional projects on internal parasites of ruminants (S-21 and W-35) provide a basis for cooperation on the project Mixed Helminth Infections. The factors which influence the Acquisition of Parasites from Pastures by cattle are being studied in the South (Regional project S-21). Georgia, Louisiana, and Puerto Rico are studying conditions such as moisture on grass blades, sunlight striking the blade, height and type of grasses. Studies conducted in Mississippi include the effect of different seasons, the type of seasonal grasses and soil sterilants on parasite larvae. Arkansas, Georgia, Louisiana, and Texas are cooperating in regional research (S-21) to evaluate factors of parasite control. Examples are management, rotation, stocking of pastures, supplemental feeding, water, soil types, drainage and shade. Georgia, Mississippi and Oregon are determining which are the best grasses or mixture of grasses for parasite control. Studies in cooperation with the USDA are in progress in the North Central Region and the Western Region on contributing conditions for Winter Coccidiosis (bloody scours). The evaluation of specific food elements, carbohydrates, fats, minerals, proteins and vitamins, are being studied in the North Central Region and Southern Region (S-21) in cooperation with the USDA to determine the Influence of Diet and Nutrition on Roundworm Parasitism. The Southern Region (S-21) and Western Region (W-35) are cooperating with the USDA to determine the Clinical and Physiological Aspects of Roundworm Parasitism in Cattle. Research studies on Anaplasmosis are in progress in the north central, southern, and western regions in cooperation with the USDA.

Industry and Other Organizations especially chemical companies, are engaged in research on the formulation of compounds and the exploration of chemicals that may be used safely as parasiticides. Most of the companies engaged in this kind of research utilize their own personnel, facilities and funds. The efforts and results of the work are generally considered as confidential since the ultimate goal of the companies is to produce saleable products. It is estimated that approximately 50 professional man-years are devoted to the work.

# REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

#### A. Ecological Factors Influencing Nematode Development.

In 1961 at the Animal Disease and Parasite Research Division's (ADP) Regional Animal Parasite Laboratory, Auburn, Alabama, the research showed that eggs are laid by Cooperia oncophora in various stages of development and their hatching times are correspondingly varied. Measurements, stages of development and appropriate comparisons with C punctata and C. curticei have been made. The shortest prepatent period was 17 days. The first 18 feet of the small intestine was found to harbor the majority of worms. The patent period of a single infection may last as long as 8 months.

In a study on immunity to the ruminant parasite, <u>Trichostrongylus colubriformis</u>, subcutaneous injection of guinea pigs with artificially exsheathed infective larvae, intraperitoneal injection with the metabolic excretions and secretions of artificially exsheathed larvae, and oral inoculations with 5,000 normal infective larvae, failed to afford any protection to the guinea pigs against subsequent oral challenge with 40,000 infective larvae.

Cooperative work at Experiment, Georgia, under a memorandum of understanding, indicated, in preliminary studies, a reduction in the number of third-stage larvae of various cattle and sheep nematodes proportional to the increase in number of viable spores of <u>Bacillus thuringiensis</u> var. thuringiensis Berliner.

In 1962, at Experiment, Georgia, under the auspices of the ADP Laboratory at Auburn, work on the ecological factors influencing nematode development, but using guinea pigs and rabbits for the tests, revealed a markedly lower number of adult  $\underline{T}$ . colubriformis was recovered from guinea pigs infected with larvae cultured at  $10^{\circ}\text{C}$  than from three other groups of guinea pigs infected with larvae reared at 15, 25, or 32°C. The hosts infected with larvae cultured at 32°C had a much higher number of larvae than those from the other three groups. However, another test using larvae of  $\underline{T}$ . axei to infect rabbits showed no difference between the number of worms recovered from the rabbits infected with larvae reared at 10 and 25°C, but a lower number of larvae was recovered from rabbits infected with larvae cultured at 32°C.

# B. Effects of Mixed Helminth Infections.

In 1961, at the Regional Animal Parasite Laboratory, Auburn, Alabama, it was found that 5.5 to 6 months-old grade Jersey calves, administered from 200,000 to 700,000 infective larvae of Cooperia oncophora, developed anorexia and an enteritis during the patent period of infection. Three groups of infected calves made average total weight gains ranging from 6.5 to 20.5 pounds less than that made by the controls; however, the differences were not significant. Blood physiology was not affected by the infections.

In 1962 this work was discontinued early in the year.

#### C. Acquisition of Parasites from Pastures.

In 1961 at the Beltsville Parasitological Laboratory (BPL), a study was made of the development of infective larvae of gastro-intestinal parasites of cattle, their migration onto herbage, and their duration thereon following deposition of feces containing eggs of these parasites at different times of the year. In general, conditions for development, migration onto the herbage, and survival were best in early Fall and in the Spring when there was an abundance of available moisture, and they were poorest in late Fall, when it was cold, and in Midsummer when it was too dry. These findings are in agreement with earlier indications of larval development and survival obtained from the numbers of worms recovered from calves that grazed off test plots.

In 1962 at the Beltsville Parasitological Laboratory, studies were continued and indicated that rotational grazing of pastures by bovines infected with gastro-intestinal nematodes apparently did not reduce levels of parasitism or improve the performance of infected animals in an experiment carried out in 1961. Two test groups initially were artificially exposed to infection equally and simultaneously. During the experiment one of the groups grazed three 1-acre pastures rotationally. The other grazed continuously on a single 3-acre pasture. The initial and each subsequent grazing period on each smaller pasture was 2 weeks, so each 1-acre plot was vacant for 4 weeks prior to each re-grazing of it. Three cycles of rotation were completed from early July to early November, when the experiment ended and the cattle were necropsied. The worms recovered at necropsy have not yet been counted, but worm-egg counts during the test indicated no substantial difference in the worm loads of the two groups.

Initial infection with the beef measles worm (Cysticercus bovis) conferred on cattle in a recent test a strong resistance (acquired resistance) to reinfection with this larval tapeworm. The results of tests to explore the possibility that age may be a factor in initial susceptibility to this parasite suggest that adult animals, with the possible exception of ones more than 5 years old, can be at least as susceptible as calves and that individual variation in susceptibility occurs.

# D. The Effect of Pasture Mixtures and Pasture Management on the Control of Internal Parasites of Cattle.

In 1961, at Experiment, Georgia, a second year feeding trial was conducted under the supervision of the Regional Animal Parasite Laboratory at Auburn. The findings reported for the first year's grazing trials were not statistically significant. The second year test was completed studying the effect of rotational grazing on different types of pasture mixtures on the level of parasitism in beef yearlings. One of 2 plots planted with temporary winter pasture was divided into 4 plots and grazed on a four-way rotational system. A third plot was also divided into four plots, each of which was planted with a different forage mixture and rotationally grazed. For the second year, the steers on the winter temporary pasture rotationally grazed harbored more worms (34,827) and exhibited a lower average daily gain (ADG) than those animals grazed continuously on the same type of forage (22,674 and 2.09 lbs.). The lower number of parasites recovered from the rotationally grazed pasture mixtures (17,149) was probably due to the excessive growth of these forages, which also reduced the ADG (1.84 lbs.), although the average stocking rate was lower than those from the other lots.

In 1962, the studies were continued at Experiment, Georgia, where the thirdyear test was completed in an attempt to study the effects of rotational grazing on different types of pasture mixtures on the level of parasitism in beef yearlings. One of the two lots planted with temporary pasture was divided into four plots and grazed on a four-way rotational system. A third lot was also divided into four plots, each of which was planted with a different forage mixture and rotationally grazed. The animals from the continuously grazed pasture had less worms (15,109) and higher ADG (3.00 lb.) than those from the two rotationally grazed pastures - 41,229 worms and 2.34 lb. from the winter temporary, and 22,299 worms and 2.15 lb. from the pasture mixture. Rotation of grazing required higher stocking rate for proper pasture utilization, which may be responsible for increased parasitism.

# E. Winter Coccidiosis (Bloody Scours) of Cattle.

In 1961, at the Animal Disease and Parasite Laboratory, Logan, Utah, fifteen calves were used in one experiment to investigate the possible transmission of passive immunity to coccidiosis caused by <u>Eimeria bovis</u> through intraperitoneal injection of concentrated serum globulin, and to observe the effects of intraperitoneal injections of sporulated occysts, or of merozoite-mucosa emulsion on the development of clinical coccidiosis. The intraperitoneal injection of sporulated occysts resulted in the development of mild coccidiosis sufficient to cause development of enough immunity to resist <u>per os</u> inoculations with 1.4 million sporulated occysts. Intraperitoneal injections with concentrated serum globulin, merozoite-mucosa emulsion, or distilled water failed to prevent coccidiosis when the animals were given 1.4 million sporulated occysts <u>per os</u>. There was no passive transfer of immunity nor alterations of the serum proteins related to any of the injections.

In another experiment sixteen yearling steer calves were used to determine how long immunity to coccidiosis persisted. Approximately 1 to 1-1.3 years after their last experimental inoculation with sporulated oocysts each of 16 calves was inoculated per os with 1.4 million sporulated oocysts. Only calves which had not previously been inoculated developed severe symptoms of coccidiosis. Severe changes in the serum protein accompanied or followed the occurrence of coccidiosis in susceptible calves. More pronounced changes in the beta and gamma globulin fractions were observed in these older calves than in the young calves. One calf, immunized about 9 months earlier and treated with sulfa drugs, developed unusually severe reductions in serum protein during the period when the susceptible calves were exhibiting severe symptoms of coccidiosis. This calf showed none of the usual symptoms seen in the other calves. This may indicate that the effects of coccidial infections are manifested in ways other than the usual diarrhea, bleeding, etc.

Thirty newly weaned calves averaging about 350 pounds each, were used to determine the effect of intraperitoneally or intramuscularly injected oocysts on the alteration of serum proteins and the development of immunity to coccidiosis. Calves injected intraperitoneally with sporulated oocysts appeared to develop resistance to per os inoculation but intramuscular injections with sporulated oocysts produced little or no immunity. Intraperitoneal and intramuscular injections with unsporulated oocysts failed to elicit the development of immunity. All of the 30 calves carried light natural infections at the time of inoculation, but per os inoculation with 300,000 sporulated oocysts produced severe effects which were sufficient to kill one calf.

In 1962 at the Logan Laboratory, the research work on winter coccidiosis was continued. No significant differences were observed in the susceptibility to coccidiosis, Eimeria bovis, infections in nursing and bucket-fed new-born calves. Uninoculated nursing control calves quickly became infected when penned with calves that had been inoculated with coccidia, but the infection was less severe. The administration of whole citrated blood intravenously to calves during the severe stage of coccidial infection, appeared to increase hemorrhage. Severe infections did not significantly alter the levels of hemoglobin, hematocrit and blood glucose, or the total serum protein.

Calves given single intraperitoneal injections with 1,000,000 sporulated oocysts of  $\underline{E}$ . bovis developed clinical coccidiosis and one of eight died. Those surviving exhibited a strong immunity to reinfection. Four calves given multiple alternate day injections of 200,000 sporulated oocysts for five injections, exhibited symptoms over a longer period than calves given a single injection. One of the four calves died, but the three survivors exhibited stronger immunity to reinfection than did any of those in the other groups.

Infection was not manifested in any of another group of calves that were injected intraperitoneally with sporulated oocysts of  $\underline{E}$ . bovis. This may indicate that the former injections were given into the intestinal tract instead of into the peritoneal cavity. Two calves injected intraperitoneally with x-ray irradiated oocysts that had been exposed to 60,000 r by a Westinghouse Quadrocondex machine did not develop signs of infection, or any immunity. Two calves given oral inoculations with irradiated oocysts showed a mild infection but exhibited immunity to reinfections.

There were no changes in the serum electrolytes, potassium or sodium, during prepatent or patent periods, or significant changes in the sera of those calves that survived the infections. Immediately before death, from coccidiosis, the blood serum potassium was elevated to 7-8 mEq/1 while the sodium levels were reduced to 90-100 mEq/1.

In 1962, at the Montana Veterinary Research Laboratory, Agricultural Experiment Station, Bozeman, under a cooperative agreement with the USDA, research work was conducted on winter coccidiosis. Observations were made on the comparative morphology and sporulation time of Eimeria ellipsoidalis, E. bovis, E. auburnensis, E. cylindrica, E. zurnii and E. brasiliensis. An attempt was made to provide criteria for differentiation of these species, and to clarify some of the apparent inconsistencies in the taxonomy of the cattle coccidia. A progressive increase was shown in oocyst length and width from E. cylindrica through E. ellipsoidalis, E. bovis, and E. brasiliensis. Sporulation time at 20 and  $30^{\circ}$ C was also distinct.

The occurrence of coccidial strains resembling <u>E</u>. <u>cylindrica</u> either morphologically or with respect to sporulation time, but differing markedly in other respects, was demonstrated in naturally infected beef calves. It is important that valid criteria be provided for differentiating cattle coccidia, since the pathogenicity of the various species differs greatly.

#### F. Influence of Diet and Nutrition of Cattle on Roundworms.

In 1961, at the Beltsville Parasitological Laboratory, Beltsville, Maryland, two comparable groups of calves were fed different levels of the same diet. Half of each group was infected with equal numbers of the same kinds of gastrointestinal helminth parasites. The infected calves on the lower level of feed consumption became more heavily infected, and, relative to their respective controls were, in general, more adversely affected by the parasitism than those on the higher level. Replication is necessary before generalization is justified.

In 1962, these studies were continued at the Beltsville Parasitological Laboratory. The results showed the efficiency of feed utilization by calves on two different levels of feeding was markedly reduced by moderate infection with gastrointestinal nematodes. Efficiency was also affected by the level of feeding and was greater at the higher level. However, the reduction in efficiency caused by the parasitism was about 8 times the difference due to feeding level. Calves on the higher intake were less severely affected by the parasitism than those on the lower level. In mild infections the higher feeding level only slightly enhanced the ability of the calves to cope with the infections and efficiency of feed utilization was affected about equally by parasitism and feeding level.

# G. Artificial Propagation of Protozoan Parasites.

In 1961 at the Beltsville Parasitological Laboratory, work was continued to develop a defined medium for the in vitro cultivation of Histomonas meleagridis, the causative agent of blackhead. This parasite was successfully propagated for the first time free of demonstrable bacteria in modified tissue culture media. Bacteria from the ceca of turkeys, heretofore considered important and routinely used in cultures of Histomonas, were successfully replaced by a variety of fresh hamster tissues enriched with metal ions, without loss of infectivity of the parasites. Histomonads grown in these tissue-containing media were capable of infecting young chickens when inoculated by rectum, whereas organisms grown in media enriched with bacteria from the ceca of turkeys were unable to do so.

Histomonads were grown in media devoid of cream, but containing cholesterol, cholesterol esters or a commercial steroid preparation which demonstrated for the first time that one of the growth factors for Histomonas is a steroid. It was found that certain fatty acid esters of cholesterol promote good histomonad growth, thereby indicating that another growth enhancing compound is probably a lipid.

Primary requisites to artificial cultivation of parasites are (1) freedom from contaminating organisms, and (2) an adequate supply of the infective stages of the parasite. With regard to coccidia, both these problems were solved.

Sterile suspensions of <u>Eimeria acervulina</u> were obtained with antibiotics. Sporocysts were released from oocysts by aseptic grinding with a mortar and pestle, and excystation of sporozoites from sporocysts was produced by treatment with sterile trypsin and chicken bile. A l cc suspension containing as many as 3 million motile sporozoites—more than enough—was obtained by use of trypsin and chicken or turkey bile. Excystation occurred within 5-10 minutes and 90-95 percent excysted within 1 hour.

In 1962 research workers at the Beltsville Parasitological Laboratory reported that cholesteryl palmitate and stearate were used successfully as replacements for cream in artificial cultivation of the protozoan parasite, Histomonas meleagridis. Growth factor s) for this parasite, provided by certain bacteria normally associated in the host, and grown in nutrient broth, appear to be intra-cellular. This factor(s) can be inactivated by subjecting the bacteria to temperatures above 56°C and below freezing, but can be regenerated by returning the bacteria to their normal culture temperature.

Bacteria grown in a variety of media other than nutrient broth have failed to sustain histomonad growth. A modification of a commercial tissue culture medium, known as "199", in which histomonads will grow after the addition of cream from cow's milk, was found capable of supporting histomonad growth even after being frozen for more than 2 months.

# H. Host-Parasite Relationships of Coccidia.

In 1961, at the Regional Animal Parasite Laboratory, Auburn, Alabama, studies on histochemical staining revealed (a) PAS reactions were positive in E. alabamensis in oocysts in tissues and in macrogametocytes. Heavily parasitized host cells, as well as mature schizonts, ceased to be PAS positive; (b) Glycogen was found in macrogametocytes of E. alabamensis and E. zurnii, as well as in some mature schizonts of E. zurnii and E. bovis; (c) Collagen fibers and keratin were found in the outer membranes covering the macroscopic schizonts of E. bovis; (d) polysaccharides, DNA and protein were studied in macroscopic schizonts of E. auburnensis and during various times in the life cycle of E. ahsata in sheep.

An acid stain by Gray, et al, using Celestine Blue B, ferric alum, glycerol and sulfuric acid was found to be the equivalent to iron hematoxylin stain, even though only l minute is required for staining. Intermediate stages of coccidia in sections of cattle intestines were stained by this method.

The Lotze method of excysting oocysts of coccidia in vitro by using overnight exposure to lipase (steapsin) solution and then additional exposure to fresh bile was confirmed at the Regional Laboratory. Living sporocysts and sporozoites of E. ahsata, freed by this method, were measures.

In 5 of 6 calves inoculated with oocysts of <u>Eimeria bukidnonensis</u>, the prepatent period was from 11 to 16 days and the patent period from 4 to 6 days. Three calves showed signs of clinical coccidiosis.

Oocysts of Eimeria auburnensis, stored in 2% potassium dichromate solution, were still viable after 3.5 to 4 years. Schizonts of  $\underline{E}$ . auburnensis, ranging up to 250 by  $100_u$  were found in the lower half of the small intestine of a calf killed on the 12th day after inoculation. Most were embedded in the mucosal layer instead of in the center of the villi as is common in  $\underline{E}$ . bovis.

In 1962 studies were continued at the Auburn Laboratory. The results of several tests were (a) Schizonts were found in the middle and posterior third of the small intestine of calves killed 12 and 14 days after they had been inoculated with pure cultures of oocysts of Eimeria auburnensis. The schizonts ranged from 78u to 250u long by 78u to 150u wide. (Sample mean 92u by 139.9u). They were usually located deep in the lamina propria near the muscularis mucosae instead of in the villi where most schizonts of E. bovis are found. The schizonts of E. auburnensis resemble the previously described large microgametocytes of this species, but were distinguishable morphologically and by histochemical stains. The microgametocytes were much larger than previously reported; one measured 91u by 287.5u.

- (b) Calves were killed 4, 11, 15, and 25 days after inoculating with Eimeria bukidnonensis. Studies of the tissues revealed sporozoites in sections of small intestine 48 ft. above the ileocecal valve, at 4 days. At 11 days, a young schizont was found at C + 12. Nothing was found at 15 but, at 25 days, an oocyst was located at C + 1.
- (c) The following mature endogenous stages of <u>Eimeria zurnii</u>, <u>E. alabamensis</u>, and <u>E. bovis</u>, coccidia of cattle, were periodic acid Schiff ("PAS") positive: merozoites, microgametocytes, traces of cytoplasma and plastic granules of macrogametes and oocysts. Immature forms of the same stages were usually PAS negative.
- (d) Celestin Blue B, Acid Fuchsin, and Orange G gave very good contrasting colors to various endogenous stages of <u>Eimeria</u> <u>zurnii</u> in sections of cattle intestines.
- (e) In two tests on excystation of oocysts of <u>Eimeria ahsata</u>, a solution of chenodesoxycholic acid, a purified bile extract, did not induce liberation of sporozoites. Sodium desoxycholate, 0.5%, following lipase exposure, released 20 percent.

# I. Ecology and Immunology of Lungworms.

In 1961, at the Beltsville Parasitological Laboratory, research showed that double vaccination of calves with x-irradiated larvae of the cattle lungworm usually offered some degree of protection against challenge exposure with this parasite. The immunization was accomplished with larvae exposed to 40,000 roentgens at 4 different rates - 100, 200, 400, and 1,200+ roentgens per minute. The larvae exposed at the lowest rate were reared and irradiated by a commercial firm. The rate of x-ray application did not materially affect the invasive powers of the larvae in mice and guinea pigs. However, challenge exposures produced the least pulmonary distress in calves given orally larvae

irradiated at the lowest rate. Also, these calves eliminated negligible numbers of first-stage larvae, had the least amount of lung damage at postmortem, and no worms were grossly observed at necropsy.

In 1962, at the Beltsville Laboratory (BPL) it was found that double oral vaccination with x-irradiated cattle lungworm larvae conferred resistance to infection with this parasite on 2 of 4 pairs of test calves. At necropsy about 1 month after challenge exposure to infection with normal larvae, each of these pairs yielded fewer worms than any of 4 control calves. One calf was vaccinated with larvae irradiated at a rate of 100 r/min., the other with larvae irradiated at 1,200+ r/min. The remaining two pairs of calves were vaccinated with larvae irradiated at intermediate rates. One of these pairs was highly susceptible, the other possibly slightly resistant to infection on challenge. An adequate explanation for these differences in results is not presently available.

Oral vaccinations with larvae of a sheep lungworm appeared to be only partially successful in immunizing calves against the cattle lungworm.

# J. Clinical and Physiological Aspects of Roundworms.

In 1961, at the School of Veterinary Medicine, University of California, Davis, under a cooperative agreement with the USDA, Ruelene, an organic phosphate with excellent anthelmintic properties, was found to be of greatest value when administered orally. When poured on the back, or injected intraperitoneally in cattle, anthelmintic activity was unsatisfactory. Iodine-free phenothiazine was found to have a slightly greater anthelmintic action than N.F. phenothiazine. The increased activity, however, was too small to account for the difference previously found between purified and N.F. phenothiazine.

In 1962, cooperative research at the California laboratory, showed that experimental Micellar phenothiazine is no more effective than good grade National Formula, but is much better than older commercial preparations. To obtain satisfactory anthelmintic action with phenothiazine, it is necessary to consider specific surface, purity as well as total dosage. The use of dosage recommendations based on dose area per kilogram body weight will probably give satisfactory results. The interaction of phenothiazine and certain organophosphates when utilized as anthelmintics was found to be additive rather than synergistic.

Hypoalbuminemia in heavily parasitized cattle was found to be due to reduced synthesis and only in terminal cases was an increased catabolism observed. Preliminary ferrokinetic studies in similar animals indicated that the anemia may result from bone marrow by poplasia and marrow hemolysis.

# K. Investigations of Trichomonad Parasites.

In  $\underline{1962}$ , at the Regional Animal Disease Laboratory, Logan, Utah, limited research was conducted on the new project since it was necessary to acquire several cultures of  $\underline{\text{Trichomonas foetus}}$  from different areas of the United States. One culture was obtained from  $\underline{\text{E}}_{ng}$ land. Rabbits were used for antibody production since they are not known to harbor  $\underline{\text{T}}$ .  $\underline{\text{foetus}}$ . However, it was found that rabbits do not tolerate a long series of injections very well. Best results were obtained for separation of rabbit-serum fractions by unidimensional starch-gel electrophoresis using a voltage drop of 6 volts per centimeter at room temperature over a period of 20 - 22 hours.

In 1962, at the Utah Agricultural Experiment Station, Logan, under a cooperative agreement with the USDA, research workers isolated a pentatrichomonad from the rumen and cecum of calves. The forms of the pentatrichomonads from both locations appear to be identical.

# L. Host-Parasite Relationship of Intestinal Worms Cooperia spp.

In 1962, at the Regional Animal Parasite Laboratory, Auburn, Alabama, progress was made on this, a newly instituted, project of research. It was found that calves 6 to 7½ months old, were severely affected by oral inoculation with 350,000 Cooperia pectinata infective larvae. The infected calves made an average weight gain of 3.8 pounds while the controls averaged 45.3 pounds. The clinically affected calves developed a pronounced hypoglycemia concomittant to the period of anorexia. The pathogenicity of this species is essentially as severe as that of the related form Cooperia punctata, and both species are much more pathogenic than C. oncophora.

The life history of <u>Cooperia pectinata</u>, an intestinal worm of cattle, is direct, requiring from 14 to 17 days to develop from the ingested infective larva to the sexually mature adult. Its rate of development is intermediate between that of <u>C. punctata</u> and <u>C. oncophora</u>. Attempts to produce hybrid nematode crosses between <u>C. oncophora</u> and <u>C. pectinata</u> were initiated. Fourth-stage female <u>C. oncophora</u> with fourth-stage male <u>C. pectinata</u> and fourth-stage male <u>C. oncophora</u> with fourth-stage female <u>C. pectinata</u> were successfully transferred to the small intestines of laparotomized helminth-free calves. Eggs in these calves' feces developed to infective larvae in culture. Photomicrographs were made of a male <u>C. pectinata</u> and a female <u>C. oncophora</u> in copula. The progeny are being studied. Chromosome preparation and staining techniques have been developed for use in this study.

A pilot study was made of the histochemistry of <u>Obeliscoides cuniculi</u> and of <u>Ostertagia ostertagi</u> and of the histochemical pathology of infection by worms of the latter species in calves. Histochemical techniques used have been Best's carmine stain, the periodic acid-Shiff reaction, the ferric mannitol technique, the Alcian blue technique, the iodine method of Nielsen, Okkels, and Stockholm (loc. cit.), and the calcium-cobalt method of Gomori for phosphomonoesterase I.

Best-positive material (glycogen) was found in the intestinal walls, ovary, and musculature of the body wall of Obeliscoides cuniculi. PA/S-positive material was detected in the cells of the digestive tract, musculature and chords of the body wall, gonads, and pseudocoelom of these worms. After treatment with malt diastase, Best-positive material is completely absent, and PA/S-positive material is absent from gonads, intestinal cells (but not the surface of the lining membrane), and musculature of body wall. Other histochemical techniques were not used with Obeliscoides.

 $\underline{\underline{In}}$  vitro tests using the Goodey skin penetration technique indicated that  $\underline{\underline{T}}$ .  $\underline{\underline{axei}}$  and  $\underline{\underline{T}}$ .  $\underline{\underline{colubriformis}}$  larvae were unable to effect skin penetration. Adult worms were recovered from the intestines of rabbits exposed to cutaneous applications of  $\underline{\underline{T}}$ .  $\underline{\underline{axei}}$  and  $\underline{\underline{T}}$ .  $\underline{\underline{colubriformis}}$ . The low yield of adult worms indicates that the larvae did not enter the rabbit percutaneously, but were probably ingested from the surface of the contaminated skin.

# M. Anaplasmosis of Cattle.

In 1961, at the Beltsville Parasitological Laboratory, research workers reported immunity studies in cattle, using an adsorbed non-viable vaccine prepared from concentrated anaplasma-infected blood, resulted in prolonged serum antibody titers to anaplasma complement-fixing antigen. The vaccinated animals did not have appreciable immunity when exposed to low doses of infected blood.

Serologically suspicious cattle, revealed by the anaplasmosis complement-fixation test, in a large commercial herd, have been studied for anaplasmosis by inoculation tests into susceptible cattle. Two of 36 such animals (5.5%) were found to be infected. These and similar previous studies indicate the need for more research on the problem of causes of CF suspects and means for separating non-specific from specific reactions.

A series of experimental transmission trials with the Rocky Mountain wood tick (<u>Dermacentor andersoni</u>) have indicated that transovarian passage of the anaplasma agent in this tick is not a common finding. It was shown experimentally that infected male ticks could transmit anaplasmosis after a period of 108 days. The relative importance of these findings to actual spread of the disease in nature is still problematical, but the information obtained to date does suggest that the male tick may be responsible for considerable natural spread of tick-borne anaplasmosis.

The fluorescent antibody technic has been applied to studies on the agent of anaplasmosis. A variety of anaplasma forms, many possessing tail-like structures, have been observed in the blood of some infected animals. Filtration experiments, using plasma from acute cases of anaplasmosis, and Millipore filters of known pore size, have established the presence of an extra-cellular form of the anaplasmosis agent. Such plasma, when passed through filters of 0.65 micron and 0.3 micron average pore size, has been found to be infective for cattle.

Field trial studies at (1) Kerrville, Texas, indicate after 2 years that isolated clean offspring of carrier cattle have remained free of anaplasmosis without the use of insect control measures; (2) Evanston, Wyoming, indicate that when negative susceptible cattle are placed on tick-infested rangelands, that anaplasmosis re-infection of the animals and pastures by indigenous Rocky Mountain wood ticks has been of slight likelihood during the past year; (3) Stoneville, Mississippi, indicate that the use of an electric-eye, automatically operated walk-through sprayer, using synergized pyrethrum, can substantially reduce the losses and spread of anaplasmosis in an infected herd.

In 1962, research on anaplasmosis of cattle was continued at the Beltsville and Kerrville laboratories, with the following results:

Transmission trials using the Rocky Mountain wood tick, <u>Dermacentor andersoni</u> Stiles, failed to demonstrate transovarian passage of the etiological agent of anaplasmosis. Ticks of this species, collected from an infected area in Wyoming, did not transmit anaplasmosis when tested on known susceptible calves. A colony of these ticks was established and stage to stage (nymph to adult) transmission of anaplasmosis was accomplished. <u>D. andersoni</u> male ticks transmitted the disease after they were held for 197 days following feeding on an acute case of anaplasmosis.

The morphological variations of <u>Anaplasma marginale</u> Theiler, as observed in infected erythrocytes by electron microscopy and immunofluorescent methods, have been studied. The forms observed by both techniques included those with and without projections or sac-like structures. It was determined that the projection of the <u>Anaplasma</u> was a valid structure and not an artifact. Ticks feeding on highly parasitized blood were found to eliminate these structures in their excreta.

Serological studies were done to compare the standardized complement-fixation (CF) serum reaction of infected animals with reactions produced by serums in a capillary agglutination (CA) test. It was found that serums preserved with a low concentration of phenol as used in the CF test did not give satisfactory results in the CA test. Although there was close agreement between results of CF and CA tests, the comparative accuracy of the two testing methods was not definitely established. It was found that some CF antigens would agglutinate in the presence of immune sera from infected animals.

The causative agent of anaplasmosis was frozen by a slow-freezing technique but failed to survive prolonged cold storage even though it did survive freezing and storage at -60°C for a 24-hour period.

Experimental field control studies demonstrated a protective effect from feeding low levels of Chlortetracycline to susceptible cattle. The natural course of anaplasmosis in a dairy herd in Louisiana is being studied to evaluate the effect of the disease on milk production. Rocky Mountain wood ticks in Wyoming are being investigated as natural transmitters of anaplasmosis.

The anaplasmosis research herd, Kerrville, Texas. The objective set in 1958 for this project has been achieved by using the complement-fixation test to identify reacting animals, isolation of reactors from negative cattle by a wire fence, strict antisepsis in all handling of the animals, a minimum of arthropod control, the original reactor herd of 38 cows and a bull eventually replaced, retention of negative heifers of that herd, and by a negatively reacting herd of 46 females and a bull. Once isolation of the heifers was accomplished, there were no breaks with the disease among them.

Entomology Research Division cattle. Regular blood samples are obtained from the main herd and from cattle imported from other States for research purposes. The disease continues to spread among these cattle and clinically recognizable cases of anaplasmosis were rather frequent during the year. Since antisepsis is routinely practiced for the blood sampling, the transmission is accomplished either by arthropod vectors or some other operation.

In 1962, a project on Investigations on Anaplasmosis, Piroplasmosis and Babesiellosis of Cattle was initiated under a PL 480 grant to the School of Veterinary, Montevideo, Uruguay. No report on progress was submitted for the quarter ending June 30, 1962.

In 1962, a project on Investigations on the Pathogenesis of Lesions Produced by the local Leech, <u>Limmatis nilotica</u>, was initiated under a PL 480 grant to the Hadassah Medical School, Hebrew University of Jerusalem, Israel. No report on progress has been made.

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# INFECTIOUS AND NONINFECTIOUS DISEASES OF SWINE Animal Disease and Parasite Research Division, ARS

Problem. Profitable swine production depends largely on the ability to control diseases. Swine diseases cause losses estimated at more than \$200 million annually. In order to control and eventually eradicate these diseases, a thorough knowledge of causes, diagnostic procedures, preventive procedures, and treatments is required. Although a great deal of excellent research has been and is being accomplished, a vast amount of research is still required to obtain this knowledge. At present, the causes of several important swine diseases are unknown or incompletely understood. Extensive fundamental research on swine diseases is essential to the welfare of the swine industry.

#### USDA PROGRAM

The <u>Department</u> has a long history of swine disease research. For example, research on hog cholera was initiated in 1884. Research on this and other important swine diseases is a continuing long-term program. Modern research techniques in the areas of biochemistry, biophysics, pathology, microbiology, pharmacology, physiology, and immunology, are being applied to swine disease problems. Research is being conducted on the following diseases at the designated locations.

The <u>Federal</u> scientific effort devoted to research in this area totals 22.3 professional man years. This effort is divided among sub-headings as follows:

Hog Cholera 8.1 at the National Animal Disease Laboratory, Ames, Iowa, the Florida Hog Cholera Research Station, Live Oak, Florida, and under a cooperative agreement with the University of Illinois.

Atrophic Rhinitis 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Transmissible Gastroenteritis 3.6 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with Purdue University and the University of California, and a memorandum of understanding with the University of Illinois.

Erysipelas 3.6 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the Department of Biochemistry, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey.

Brucellosis 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 18.1 professional man years divided among sub-headings as follows: death losses in young pigs 5.6, hog cholera 3.4, atrophic rhinitis 2.0, transmissible gastroenteritis 2.7, erysipelas 0.7, brucellosis 2.4, other diseases (salmonellosis, vesicular stomatitis, etc.) 1.3. Minnesota, Pennsylvania, Kansas, Ohio, Missouri, and Georgia are conducting studies on hog cholera. Indiana, Iowa, Nebraska, and Michigan are working on atrophic rhinitis. Illinois, Nebraska, Indiana, and Michigan are working on transmissible gastroenteritis. Indiana and Georgia are working on erysipelas. The research on death losses in baby pigs was carried out under regional research project NC-13.

Industry and other organizations. Veterinary biological and pharmaceutical companies conduct research on the development and improvement of immunizing agents, drugs and antibiotics for the treatment and prevention of swine diseases. Preventive vaccines for hog cholera, erysipelas, and leptospirosis are among the products being investigated. Drugs and antibiotics for treatment of respiratory and enteric infections are being developed. Estimated annual expenditures are approximately 50 professional man years.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

#### A. Hog Cholera.

In 1961, hog cholera research at the National Animal Disease Laboratory, Ames, Iowa, was conducted in the following phases:

Occurrence of hog cholera after immunization. Investigations were made to determine the cause of cholera outbreaks in swine that had been vaccinated. Seventeen field specimens were studied. Three had no virus present and 14 had virus which were classified as follows: 3 variant viruses; 9 regular viruses, and 2 immunizing viruses. Seven of the regular virus were of such low virulence that all the pigs recovered after being sick 3 to 7 days. Salmonella and Pasteurella isolated from sick farm pigs caused cholera-susceptible and experimental pigs to become sick or die when infected simultaneously with modified hog cholera vaccines.

Propagation of hog cholera virus in vitro. Hog cholera hyperimmune serums prepared in pigs and rabbits, and serums from noninfected pigs and rabbits, were fractionated with Rivanol and ammonium sulfate. The gamma globulin fractions were conjugated with fluorescein isothiocyanate. Infected PK cells (a transmissible line of swine kidney cells), primary swine kidney cells, primary swine testicle cells, primary swine bone marrow cells, and noninfected cells of the same origins, were exposed to the labeled antibody. Smears prepared from the peripheral blood and lymph nodes of swine acutely sick with hog cholera, were treated the same as cultures. None of the infected cultures nor the smears were suitable for demonstration of specific immunofluorescence since the nonspecific reactions were of the same amount and intensity in both the infected and noninfected cells.

Studies were conducted on a PK swine kidney cell line persistently infected with an agent that immunized against hog cholera virus. The transmissible line of swine kidney cells (PK) was received from a commercial company. Apparent rapid modification of virulent virus used as inoculum and marked fluorescence of noninfected cultures suggested that the PK cells were already carriers of an agent antigenically related to hog cholera virus. The presence of the contaminating modified agent was demonstrated by immunizing susceptible pigs against hog cholera with supernatant fluid from an uninoculated PK cell culture. Confirmation that the cell strain was contaminated prior to its arrival at the Hog Cholera Station was demonstrated by immunizing susceptible pigs with another PK cell culture from the original source, immediately after receipt at the Station. It was not possible to eliminate this immunizing agent from the cell strain by using hog cholera hyperimmune serum in the tissue culture medium.

Immunizing properties of hog cholera vaccines. Investigations were made of the factors influencing the potency and immunizing ability of hog cholera vaccines in individual pigs and groups of pigs. One lot of virus (1840 ml) was made and tested for variant characteristics, but none were found. It had a titration of 2,500,000 minimum lethal doses per milliliter. This virus will be used for exposure of swine in various kinds of experiments and supplying experiment stations doing research on hog cholera.

An experiment was carried out to show the relative susceptibility to hog cholera virus of inbred lines of Hampshire, Duroc, Poland China, Yorkshire Landrace, and Chester-white pigs. Four of 6 Hampshires died; 5 of 6 Durocs died; 1 of 6 Poland-Chinas died; 1 of 5 Yorkshires died; none of the 6 Landrace died, and 1 of 2 Chester-whites died. The Durocs and Hampshires seem to be the most susceptible.

Crystal violet glycerol (CVG) vaccine, Sn. 116, that was made in 1959 and used to vaccinate farm herds in 1960, was re-tested and found to give 78 percent protection. The percent protection had not changed since being tested a year previous. Another vaccine, Sn. 117, consisting of 124,000 ml. was prepared in 5 sub-lots. Some of the sub-lots and the final pool were tested in Florida. All protection tests proved satisfactory. Part of this vaccine, 112,500 ml. was shipped to the Live Oak Station to be used in an experimental eradication trial in Lowndes County, Georgia.

CVG vaccination of 123 herds on 60 farms, composed of 12,088 pigs, was carried out in 1960-61. The immunity of a total of 486 pigs was challenged, 244 head at 1 month, and 242 head at 3 months after vaccination. The total percent protection for the year was 69.22. Last year it was 64.7. Death loss was 13.99 percent or 1.87 percent more than last year, although there was an increase of 13.13 percent in the number of normal pigs and a decrease of 7.78 percent and 7.55 percent in the slight and severe reactors, respectively. Two lots of vaccine serials 115 and 116 were used in this year's vaccination. Two viruses, serials 313 and 316, were used for the challenge of immunity.

Those herds which had little or no protection from single vaccination had 100 percent protection when given 2 doses of vaccine 1 month apart. Twenty-five sows, given 2 doses of CVG vaccine 1 month apart, had no reaction to exposure with virulent virus at 12 or 18 months after the last dose of vaccine.

The work on the reactivation of the viruses in modified vaccines was completed and published. Six modified viruses passed serially through pigs, all regained virulence so that they either made the pigs sick or caused death. Three of these viruses usually were transmitted from the injected pigs to the contact control pigs. The transmissibility by contact of the other 3 vaccines was not tested.

Some viruses of lapine origin regained their virulence in 6 passages. Other viruses of lapine origin required 19 passages before they produced sickness in susceptible pigs. A virus of porcine origin caused a severe reaction in pigs on the first passage when no serum was given with the virus. It increased in virulence on subsequent passages.

The addition of 0.15 percent of B-Propiolactone to hog blood that contained cholera virus and incubated at room temperature for 1 hour, killed the virus so that no reaction was produced when injected into susceptible pigs. These pigs developed sufficient immunity to survive a 2 ml. dose of virulent hog cholera virus. However, they were sick from 2 to 5 days. Similar results were obtained by adding 0.16 percent B-Propiolactone to blood containing hog cholera virus and incubating at room temperature for 30 minutes, or by adding 0.32 percent B-Propiolactone to blood containing hog cholera virus and incubating for 30 minutes.

Six accession lots of pigs had varying degrees of protection when 6 pigs from each lot were injected with graded doses of the same serial of vaccine and challenged with the same virus. Some lots had 100 percent protection while other lots had little or no protection.

Another lot of 42 pigs, composed of 6 breeds of pigs, seven in each breed, were all given graded doses of the same vaccine to determine if a genetic factor had any influence on their inability to develop immunity. All of the Poland China pigs died when challenged 21 days after vaccination. All except one of the Landrace died. All of the Chester-Whites survived. Four of the 6 Hampshires survived. Four of the Yorkshires survived, and 4 of the Durocs survived. This seems to indicate that there is a difference in breeds as to their ability to develop immunity.

In 1962, at the National Animal Disease Laboratory, immunizing studies were continued with the vaccination of a farm herd of 450 pigs, composed of three age groups, with crystal violet glycerol vaccine. One month later part of each group was given a second injection of CVG. Six months later representatives from each age group with single and double vaccinations were given hog cholera virus. Older pigs, vaccinated after weaning, were better protected than younger age groups. Double vaccination increased the protection 59 percent in the older group and 29 percent in the younger group. Double vaccination shortened the recovery period from 10 days to 8 days.

White cell counts of these animals showed that the rate and extent of drop in numbers of cells following virus challenge was about the same for single and double vaccinates of all age groups. The return to normal cell count was two days faster in the double vaccinates and the increase in number of cells was greater in the double vaccinates. The rate of recovery to a normal condition is related to the increase in leucocytes. Bacteriological studies of the hogs that died showed no pathogenic organisms present.

One lot of Crystal Violet Glycerol vaccine, consisting of 322 liters was made in 8 sublots. It is being tested for potency.

A total of 606 agar-gel precipitin diagnostic tests for hog cholera were made. Of 414 positive tests, 79 percent were obtained with normal pancreas material and 58 percent were obtained with pancreas from hog cholera infected animals.

The Taylor test for hog cholera, when made on a selected group of 22 virus-only-treated pigs and on vaccinated-and-virus treated hogs gave clear positive tests in 65.5 percent of the animals. The test was doubtful in 31 percent of the cases and 3.4 percent negative.

Bacteriological study of the organs from 630 hog cholera-infected pigs gave isolations of organisms from 114, divided in the following proportions:

Escherichia coli 34; Staphylococcus spp. 29; Pseudomonus spp. 14;

Streptococcus spp. 13; Proteus spp. 12; Diplococcus spp. 5; Aerobacter spp. 3; Corynebacterium spp. 2; Pasteurella spp. 1, and Escherichia fruendii 1.

Field evaluation of modified live-virus hog cholera vaccines. In 1961, 30,872 pigs from 1,023 herds on 517 farms in Suwannee County, Florida, were vaccinated with modified live-virus vaccines and antiserum. Approximately 85 percent of the swine in the county were vaccinated. Lapine origin vaccine was used on 35 percent of the pigs; porcine origin on 38.4 percent of the pigs, and tissue culture origin vaccine on 26.6 percent of the pigs.

Immunity tests on 661 randomly selected market-age swine from 340 herds showed that 82.5 percent of the pigs were adequately protected. This was an increase over the 78.1 percent protection from the previous year. The increase probably resulted from the use of fresh vaccines in recent months, and it supports the hypothesis that there is an inverse relationship between the age of the vaccine and its protective potency.

There were 13 confirmed hog cholera virus isolations; 7 from non-vaccinated swine and 6 from vaccinated swine. Thus more than 50 percent of the virus isolations came from the 15 percent of the swine that were not vaccinated. All 13 positive cases occurred during the first half of fiscal year 1961. This was a significant decrease from the 24 isolations in 1960. It appears that the high level of vaccination coverage has kept the disease well under control.

An investigation of immunization failure is centered around possible genetic resistance to immunization. Breeding stock was obtained from a herd from which only 10 of 19 animals were adequately protected by vaccination. In

preliminary studies, 2 of 6 first-generation pigs were susceptible to virus challenge given 104 days after vaccination with modified live-virus vaccines. One of 2 second-generation pigs was susceptible when given an immunity challenge 80 days after vaccination.

Titration of 6 different vaccines were carried out to determine minimum protective doses. There was a great variation of potencies among the vaccines tested. Further work is needed on the titration of vaccines as soon as possible after production and at 6-month intervals until the expiration date in order to plot a reliable immunogenicity retrogression curve.

In 1962 in Suwannee County, Florida, 9,307 swine were vaccinated against hog cholera with lapine origin vaccine, 295 of them were challenged, and 260, or 88.1 percent were adequately protected. A total of 10,931 swine were vaccinated with porcine origin vaccine, 321 of them were challenged and 278, or 86.6 percent were adequately protected. A total of 9,622 swine were vaccinated with tissue culture vaccine, 191 of them were challenged and 165, or 86.4 percent were adequately protected. The totals for all types combined were 29,860 swine vaccinated, 807 challenged, and 703, or 87.1 percent adequately protected.

Porcine origin vaccines recovered from a low percentage of adequately protected pigs of 61.6 percent in fiscal year 1960 to a high of 86.6 percent in fiscal year 1962. The average age of porcine origin vaccines (shelf-life) in fiscal year 1960 was 564.4 days (more than 18 months) whereas the age of the same type of vaccine in fiscal year 1962 was only 206.3 days (less than 7 months). The poor showing of porcine origin vaccine in fiscal year 1960 involved 8 serial numbers from 4 manufacturers. The recovery of this vaccine in fiscal year 1962 involved 6 serial numbers from 3 manufacturers.

The efficacy of all porcine origin vaccines during the past 6 years shows approximately the same pattern as described above. The average percentage of adequately protected pigs was 88.5 when the vaccines were less than 1 year old. When the vaccines were between 1 year and 18 months of age, the percentage of adequately protected pigs dropped to 80.3, and when the vaccines were more than 18 months of age, this figure was 57.9 percent. Lapine origin vaccines and tissue culture vaccines showed similar correlative declines but they were not as marked and not as early. With lapine origin vaccines, 88.5 percent of pigs were adequately protected if the vaccines were less than 18 months of age, whereas, this figure was only 80.9 percent when the vaccines were more than 18 months of age. (The minimum acceptable percentage of adequately protected pigs is 80.0 percent). With tissue culture vaccines, 91.9 percent of pigs were adequately protected when the vaccines were less than 18 months of age, and 87.8 percent were adequately protected when the vaccines were more than 18 months of age, and 87.8 percent were adequately protected when the vaccines were more than 18 months of age.

During fiscal year 1962, a field trial study was started in Lowndes County, Georgia, in cooperation with the Animal Disease Eradication Division of ARS and the State of Georgia, under the terms of a Memorandum of Understanding. This arrangement was similar to the one in Suwannee County, Florida, to determine the efficacy of inactivated and killed vaccines. Formal agreements were entered into with 703 swine owners, which is about 97 percent of the total swine owners in the county.

During fiscal year 1962, in Lowndes County, Georgia, a total of 23,899 swine were vaccinated with Boynton's Tissue Vaccine (BTV), and experimental Crystal Violet vaccine (ECVG) and a commercial CVG vaccine (CCVG). A total of 5,289 swine were vaccinated against hog cholera with BTV, 9 of them were challenged and all 9 were adequately protected. A total of 9,963 swine were vaccinated with ECVG vaccine, 50 of them were challenged and 49 were adequately protected. A total of 8,647 swine were vaccinated with CCVG vaccine, 10 of them were challenged and all 10 were adequately protected. Additional swine receiving each type of vaccine will be challenged when they reach market age.

Three positive cases of hog cholera in Suwannee County, Florida, were disclosed in non-vaccinated, farm-raised swine on 2 farms and in vaccinated, farm-raised swine on 1 farm. In Lowndes County, Georgia, hog cholera was confirmed in purchased, non-vaccinated swine on 1 farm.

In other tests, in Suwannee County, Florida, it was shown that 2 doses of inactivated vaccine administered 30 days apart, imparts almost 100 percent immunity, even if serum is administered simultaneously with the first dose.

Development of a rapid diagnostic test for hog cholera. In January 1962, this work, carried out at the University of Illinois, under a cooperative agreement was initiated. Preliminary investigation indicates that a hemagglutination test has promise as a rapid diagnostic test for hog cholera.

The relationship of hog cholera to bovine mucosal disease. In 1962 work was carried out under a cooperative agreement on the mucosal disease-virus diarrhea complex of cattle at the College of Veterinary Medicine, Iowa State University. The experimental results indicate that the Sanders mucosal disease agent does not give uniform protection to swine against virulent hog cholera virus. The results definitely indicate that the Sanders Mucosal Disease Agents affords protection in some of the swine against virulent hog cholera virus.

One experiment, consisting of 8 pigs, has been conducted in this area of study so far. The pigs were divided into 4 lots of 2 each. Two pigs were inoculated intravenously; 2 subcutaneously and 2 intramuscularly with Sanders Mucosal Disease Agent. Two pigs remained as controls. The only evidence of response to the inoculation was a drop of about 50 percent in the total leukocytes between the 4th and 6th days postinoculation.

Nineteen days later all 8 pigs were inoculated subcutaneously with virulent hog cholera virus. The 2 control pigs developed clinical evidence of hog cholera 3 days postinoculation and were destroyed in extremis on the 9th and 11th days postinoculation. Typical lesions of hog cholera were observed at necropsy.

One of the pigs which had been inoculated intravenously with Sanders agent developed clinical symptoms of hog cholera on the 2nd day post-challenge and was destroyed in extremis 15 days later. Lesions of hog cholera were evident at necropsy. The second pig to receive the intravenous inoculation of Sanders Agent displayed a mild pyrexia, depression, and weakness in the rear quarters between the 4th and 14th days post-challenge. After this period the pig showed no evidence of illness and apparently returned to normal health.

Of the two pigs that received the Sanders Agent <u>intramuscularly</u>, one showed little evidence of infection following the hog cholera challenge, the other pig, however, was quite ill between the 4th and 12th days post-challenge. During this period the temperature varied from normal to 106°F. The animal was very weak, depressed and ate very sparingly. After this period the pig rapidly returned to normal.

One of the two pigs that received the <u>subcutaneous</u> inoculation of Sanders Agent developed typical symptoms of hog cholera on the 3rd day post-challenge and was destroyed <u>in extremis</u> on the 9th day post-challenge. Lesions of hog cholera were evident at necropsy. The other pig to receive the Sanders inoculation subcutaneously developed symptoms 6 days post-challenge and was ill for 7 days. During this time the pig was markedly depressed, off feed and showed a variable temperature elevation. Following this period the pig rapidly returned to normal.

#### B. Atrophic Rhinitis.

In 1961, at the Animal Disease Station, Beltsville, Maryland, studies were carried out on the causative agent or agents, mode of spread, diagnosis, and control of atrophic rhinitis. Progress in the atrophic rhinitis (AR) project is developing methods of inoculation and storage of inoculum pools during this period was satisfactory, and data obtained further substantiated findings of previous years. The contributions were (1) frozen inoculum will remain viable for at least 8months, whereas previous tests of frozen inoculum showed viability for only 4 months; (2) the material can be diluted out at least 40 times and still remain infective. Previous tests were made only with dilutions of 1:3.5; (3) the transmissible nature of AR infected nasal turbinate mucus membrane tissue was clarified by experiments testing the noninfected materials and conditions associated with positive transmission. Air alone, saline alone, or normal masal turbinate mucus membrane tissue did not produce atrophy of the turbinates when instilled into the nasal cavity of susceptible pigs by means of the DeVilbiss atomizer with an electric pump at 13 pounds pressure; (4) the atrophic rhinitis susceptibility of pigs 14 to 25 days of age was similar to previous years experimental results with pigs under 14 days of age, and a few swine over 2 months of age, when tested, were susceptible to atrophic rhinitis exposure; (5) streptomycin or a combination including streptomycin, will inhibit atrophy-producing ability when mixed with the inoculum, whereas other antibiotics tested (penicillin, polymyxin or bacitracin) will not; (6) data obtained shows lyophilization seems to be the poorest method of storage of the inoculum; (7) the use of the rhinoscope may be of some value for diagnosing AR in a herd, but is not efficient enough for the critical work required of it for research.

In 1962 work was initiated at the National Animal Disease Laboratory, Ames, Iowa. Work during the past year was associated with the hiring and training of new project personnel, testing the established AR-free swine herd for susceptibility to the disease, developing and testing new animal cage isolation equipment and testing the deep freeze stored AR material from Beltsville for viability and transmissibility to susceptible pigs. The development of closed air and sewage systems in a plexiglass Horsfal-Bauer type isolation cage, 24"x24"x36" used in 3 experiments maintained satisfactory isolation between AR infected and normal control pigs up to 100 pounds live weight for approximately 60 days. The 20 ARfree, purebred Yorkshire sows moved from Beltsville, Maryland, have provided a satisfactory source of susceptible pigs. The atrophic rhinitis frozen agent harvested and shipped from Beltsville was viable and transmissible to pigs in a 1:3.5 dilution after 16 months storage. It was transmissible to pigs in a 1:56 titration dilution after 181/2 months storage. However, the pig passage of epithelial tissue harvested from the NADL experimental pigs was negative. Swine age susceptibility tests are in progress.

#### C. Transmissible Gastroenteritis

In 1961 preliminary work at the National Animal Disease Laboratory, Ames, Iowa, was directed toward producing disease-free swine for use in controlled experiments on transmissible gastroenteritis (TGE).

In 1962 at NADL, two lines of cells, one from fetal swine testis and one from fetal swine kidney, were developed in this laboratory for use in research on TGE. Only the swine testis cells have been used thus far. Characteristically these cells grow very slowly and require 5 percent CO<sub>2</sub> in the atmosphere for optimum growth. The cells have been passed only 16 passages in the 8 months since they were started. Eight passages were made in the first 3 months and 8 passages have been made in the last 5 months.

Investigations utilizing two isolates of transmissible gastroenteritis have been undertaken. One isolate obtained from a natural field outbreak in Central Iowa, and a second isolate received from the Purdue University, were used. It is the original Indiana isolate of TGE. Both isolates were pathogenic for very young specific-pathogen-free (SPF) pigs.

The Iowa isolate was inoculated into the swine testis cells at the 8th passage of the cells. The cytoplasm of many of the cells became granular and there was a marked acceleration growth pattern of the passaged cells as compared with noninoculated control cell line. The persistently infected cells have been passed 20 times since they were infected 5 months previously. They grow well without CO<sub>2</sub> atmosphere and in Earle's balanced salt solution and 10 percent pig serum.

Positive evidence of viral invasion of the cells was obtained by feeding 2 six-day-old SPF pigs infected cells after they had been passed 15 times. The infected pigs showed no overt signs of disease, but when they were challenged, along with the control pig, with the original homologous virus isolate, only the control pig developed clinical evidence of TGE.

The Purdue isolate was passed 3 times in an established line of pig kidney cells obtained from another laboratory. There was no evidence of infection or multiplication of the virus in these tissue culture cells. Positive evidence of viral invasion of the cells was indicated when the 3rd passage of the tissue culture passed virus was fed to each of two 6-day-old SPF pigs. The virus appeared to be partially attenuated as only one pig developed signs of TGE and died. This pig had lesions suggestive of TGE. The second pig showed no signs of disease. When the remaining pig and a control pig were challenged with pig passaged homologous virus only the control pig developed clinical evidence of TGE.

In 1961, under a cooperative agreement with the University of California, at Davis, studies were conducted with 4 of 6 viral agents isolated from the intestine of young pigs suffering from an acute scouring in the field. Investigations were conducted to determine the optimum conditions under which the 4 agents could be propagated and demonstrated by tissue culture methods.

A neutralization test, using the tissue culture system, showed an antigenic relationship among the viruses obtained from pigs with scours which were serologically unrelated to a viral agent obtained from an apparently normal hog.

Investigations on the physical properties of the viruses were carried out. Exposure at 56 C for 40 minutes inactivated 90 percent of the virus but infectious material was still present after 2 hours; after 5 hours no infectious virus remained. The viruses were resistant to 20 percent ether, 1 percent trypsin, and were viable after 300 days at -20 C. None of the viruses hemagglutinated red blood cells of chickens, guinea pigs, sheep, or swine.

Preliminary trials with one of the agents obtained from the scouring pig resulted in signs of leg weakness, paralysis and definite histopathological lesions in the brain stem and spinal cord, when inoculated into 24-hour-old specific pathogen-free pigs.

In 1962, at the University of California, physical, serological and pathological studies were performed with four strains of porcine enteroviruses designated as El, E2, E3, and E4. The particle size of these agents was estimated to be 33 to 46 mu in diameter by gradocol membrane filtration. The relationship between these four strains was studied by agar gel diffusion test, and further work is now in progress.

The porcine enterovirus (PE-1 strain) isolated by the Canadians differed from our four strains as determined by the tissue culture plaque neutralization test. Pathogenesis of the four strains was studied by oral inoculation into SPF pigs. In most cases, the pigs inoculated with El, E2, or E3 strain showed signs of gastro-intestinal enteritis, and the virus was isolated from the lower intestinal tract. One of five pigs inoculated orally with the El strain showed paralysis in the hind legs and poliomyelitis lesions were demonstrated in the spinal cord by histopathological examination.

In 1961, under a cooperative agreement with Purdue University, Lafayette, Indiana, duration of immunity to transmissible gastroenteritis was tested by immunizing 6 gilts by oral exposure to virulent virus during pregnancy. In the first farrowing their pigs had morbidity of 19 percent and mortality of 12 percent. Fifteen months after the first farrowing, the sows farrowed again and on challenge of the pigs at 3 days of age, the pigs showed morbidity of 11 percent and mortality of 8 percent. Three of 4 sows conferred solid immunity to their pigs. To test the duration of the carrier state, fecal samples were taken from 35 pigs at weekly intervals for 11 weeks and tested for the presence of TGE virus. The 35 pigs were left with the sows until weaned at 6 to 7 weeks. Of 375 fecal samples, 188 were tested by inoculating individual pigs in isolation. TGE virus was demonstrated in only 10 of 35 pigs in the first week, from 2 pigs in the second week, and none in the weeks thereafter. The results are at variance with the only previous report on the subject in which pigs shed TGE virus for as long as 8 weeks after exposure.

In  $\underline{1962}$ , at Purdue University, two phases of immunity to TGE were studied. In continuing the work on duration of immunity, it was found that gilts infected as suckling pigs between 17 and 25 days of age did not retain sufficient immunity to protect their litters from our standard challenge after one year. There was some prolongation of incubation period and some diminution in death loss over controls.

Antibodies absorbed from colostrum through the gut of the newborn animal are considered to be the important mechanism of transfer of immunity from dam to young in most farm species including swine. Experiments were done in which pigs were transferred from immune sows to non-immune sows and vice versa. It was shown that immunity against TGE transferred from sow to pig is dependent upon a continuous supply of "immune" milk. Intraperitoneally inoculated anti-serum failed to protect pigs while the same anti-serum protected when fed in the milk indicating that the important site of action is probably in the lumen or walls of the alimentary tract.

Persistence of virus shedding was not studied extensively because this work requires large numbers of pigs which were in temporarily short supply. However, it was found again that three infected pigs did not shed virus after one week. Four-day-old pigs shed as much as 10,000 infectious doses of virus per gram of feces as early as 24 hours after infection.

New approaches to adapt TGE virus to tissue cultures were made this year involving kidney monolayer cultures, organotypic cultures of various visceral organs including various parts of the gut, and a modified organ culture technique patterned after Warren's technique for growing polio-virus in monkey gut. While none of these was completely successful, the method of growing explants of intestine on millipore filters is being developed and in the best cultures would at least maintain TGE virus for three to four days.

# D. Swine Erysipelas

In 1961, at the Animal Disease Station, Beltsville, Maryland, fifty pigs, farrowed by vaccinated swine-erysipelas-immune dams, were divided into 5 groups of 10 pigs each after weaning. They were vaccinated with anti-swine erysipelas serum Lot G, and commercial desiccated virulent Ery. rhusiopathiae at different mean ages after birth (59.7 to 115.7 days). All were exposed to infection 94 days after vaccination, including 14 pen-contact controls, by the percutaneous method of exposure. There was a trend of improvement in the immunizing response to vaccination as the age when vaccinated was increased. All pen contacts were susceptible. The experimental results indicated that pigs farrowed by immune dams should not be vaccinated with virulent culture and serum until after they are 3 months old.

Biochemical investigations were carried out in cooperation with the Department of Biochemistry, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey. Buffer extracts of <a href="Ery. rhusiopathiae">Ery. rhusiopathiae</a> were resolved into 5 antigens that were distinguishable on the basis of (1) chromatographic properties, (2) heat stability, and (3) reaction of non-identity in agar diffusion studies. The antigens were identified as homologous, isologous and heterologous with respect to known serotypes.

In 1962, at the National Animal Disease Laboratory, Ames, Iowa, work was carried out as follows: 1) No gross evidence of arthritis was observed in rabbits after sensitization to Erysipelothrix rhusiopathiae in combination with Fruend's adjuvant, when injected intradermally in the interdigital space and side. 2) Acetone powder was prepared from 147 liters of broth culture for the immunological and chemical characterization of the antigen components of Ery. rhusiopathiae. 3) Peak A, the type-specific antigen derived from acetone powder, is identical with the acid extracts of the cells that formed the basis for the original classification of strains of E. rhusiopathiae.

piochemical investigations were continued cooperatively with the Department of Biochemistry, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey. Ion-exchange chromatography of neutral extracts of acetone powders of Ery. rhusiopathiae strain S-192 on DEAE-cellulose columns resulted in the isolation of 5 serologically distinct antigens when assayed by the agar double diffusion technique. Peak A, the "breakthrough" peak from the column precipitated only with homologous anti S-192 serum and the antiserum to a closely related strain NF4.

During the period covered by this report, purification of the serologically active material in Peak A by chromatography, acetone fractionation, and exhaustive dialysis yielded a preparation which had all of the serological activity of the original Peak A.

Studies on the chemical composition of this type-specific antigen have indicated that it is a mucopeptide most probably derived from the cell wall of the organism. It has been demonstrated that the antigen isolated in this manner from acetone powders of the organism is identical both chemically and serologically with the antigen present in acid extracts of the organism. It was acid extracts of cells of <u>Erysipelothrix</u> which were used originally to type the various strains of the organism.

#### E. Brucellosis.

Under a cooperative agreement on bovine brucellosis, reported in Area 1, with the College of Veterinary Medicine of the University of Minnesota, studies on brucellosis in swine were undertaken in April of 1962.

Sows and boars which react to the tube seroagglutination test for brucellosis at the time of slaughter are being traced to the farm of origin. These selected herds are followed and evaluated using epidemiologic and serologic methods developed for the detection of frank and inapparent infections of brucellosis in cattle. These studies include attempts to isolate the organism from all animals which leave the herd for slaughter using culture media, guinea pig inoculation, and fluorescent antibody micropsy.

Blood has been collected from 363 sows which have been identified prior to slaughter in order that all of the animals from which blood was collected may be traced to the herd of origin. These sows are selected with the cooperation of a packing plant, and the herds of origin are located in southern

Minnesota and northern Iowa. Initial serological studies include the plate sero-agglutination test, tube sero-agglutination test, acid plate antigen test, heat inactivation test, rivanol precipitation plate sero-agglutination test and in some cases the 56°F heat inactivation test. In the herd studies, the 56° HIT and MCE tests will be included on all animals. Preliminary results indicate that the number of reactors to the plate and tube agglutination test at dilutions of 1:100 and greater are quite low; however, approximately 10% of the animals show plate or tube reactions at 1:25 or 1:50 dilutions. The results of the supplemental tests are now being evaluated. Trace-backs are in progress to locate the herds of origin of those animals which reacted.

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# FOOT-AND-MOUTH DISEASE AND OTHER EXOTIC DISEASES OF SWINE Animal Disease and Parasite Research Division, ARS

Problem. Foreign diseases such as foot-and-mouth disease, African swine fever, and Teschen disease, that occur elsewhere in the world, constitute calculable potential threats to the swine industry of the United States. Foot-and-mouth disease is of particular importance because the disease frequently occurs primarily in swine from which it spreads to other susceptible species, such as cattle and other ruminants. African swine fever, which until recently was confined to wild and domestic pigs in Africa, has spread to Portugal and Spain. The disease is of special concern because of its resemblance to hog cholera, with which it may be confused. Moreover, mortality from the disease approaches 100 per cent, and there is no specific preventive vaccine. Teschen disease, which causes widespread inapparent infections and occasional involvement of the central nervous system, is another of the foreign diseases to be guarded against. A disease indistinguishable from Teschen disease has appeared in England in recent years. Despite all precautions, any of these diseases may occur in the United States, as likely as not through the medium of modern, rapid international transportation. The Plum Island Animal Disease Laboratory is engaged in studies of foreign diseases of swine, for the purpose of developing information for increased protection of the Nation's swine industry.

#### USDA PROGRAM

The Department has a continuing long-term program involving veterinarians, biochemists, microbiologists, and pathologists, engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 5.6 professional man years. This effort is divided among sub-headings as follows:

Foot-and-Mouth Disease of Swine 1.0 at the Plum Island Animal Disease Laboratory, Plum Island, New York.

African Swine Fever 4.6 at the Plum Island Animal Disease Laboratory in cooperation with the East African Veterinary Research Organization, Muguga, Kenya, and in connection with a P.L. 400 project in Madrid, Spain, where the equivalent of \$97,550 has been made available to the Spanish Ministry of Agriculture over a 3-year period.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

Owing to prohibition of experimentation with these diseases in the United States, except at the Plum Island Laboratory, no work in this area is being done in this country at State Experiment Stations or by American industry.

#### REPORT OF PROGRESS FOR USDA

#### A. Foot-and-mouth Disease

During 1961 at the Plum Island Animal Disease Laboratory, immunological investigations of foot-and-mouth disease of swine were carried out. The response of a group of swine to infection with foot-and-mouth disease virus was determined. These studies confirmed the concept that fundamental differences exist in immune response between swine and cattle. Swine respond to infection with rapid appearance of antibody which drops to low levels within four months, permitting some of the animals to become reinfected by contact with infected animals. Vaccination of swine with formalized tissue culture vaccine has generally been unsatisfactory.

During 1962 research effort has been directed toward the development of assay procedures suitable for study of antibody in swine serum. Experimentation has been directed toward determination of the antibody response after infection. Pretreatment of swine serum with dilute solutions of formaldehyde completely removed the procomplement activity and permitted detection of FMD antibody by complement-fixing methods. This agreed with previous findings in work with African swine fever virus. In some trials, however, it has been observed that complete fixation did not occur unless normal bovine serum was included in the mixture.

#### B. African Swine Fever

In 1961 investigations were continued in Kenya in cooperation with the staff of the East African Veterinary Research Organization. Bush pigs, as well as wart hogs, have been found to be inapparent carriers of the disease. Three virus isolations were made from 76 bush pig specimens examined.

Since African swine fever has become widespread in Spain and Portugal there is even greater need for methods of differentiating ASF from hog cholera, and for a better understanding of the mechanism of infection with ASF virus and other aspects of the disease that are important in prevention, control, and eradication.

An improved and simplified method of preparing pig leukocyte cultures has made possible production of uniform cultures for routine use in assaying ASFV. These cultures are being used to measure rates of virus inactivation occurring at various levels of pH and temperature.

By alternate passages in pig leukocyte and chicken embryo cell cultures it has been possible to adapt several strains of ASF virus first to chicken embryo cells and then to a line of pig kidney cells.

The enhancement of the complement-fixation reaction by the addition of normal bovine serum has made possible the detection of complement-fixation antigen in extracts of spleen and lymph nodes from pigs infected with ASFV. Additional work is required to establish the reliability of the technique.

In preliminary studies, certain of the antigenic components, active in the agar-gel precipitin test, are destroyed by proteolytic enzymes while others are not. The antigens also vary in their heat stability.

In 1962 epizootiological studies have continued to determine the incidence of carriers of the virus among wild species of animals in Kenya. In addition, work is being conducted to determine the number of types of virus in order that a logical approach may be made to develop immunizing agents.

Work has continued to adapt various stains of ASF virus to cell cultures. Methods for adapting ASFV to cell cultures have been established and several isolates have been serially propagated in chick embryo cells and a line of pig kidney cells. There has been evidence of some modification of the virus following passage in cell cultures. More isolates will be adapted and serially propagated in cell cultures to obtain one or more attenuated strains which may serve as immunizing agents against all strains of ASFV.

Work is also continuing to develop means of propagation of quantities of ASFV by cell culture methods. Quantities of the virus will be especially useful in studies on virus inactivation and production of viral antigens. Serological investigations have continued and it has been shown that swine which survive the acute stage of ASF develop complement-fixing antibodies against antigens in infected tissue culture. The development of a means of serological diagnosis is a significant advancement and one which should supplement diagnosis by the hemadsorption test which was developed several years ago. Diagnosis, however, by serological methods would permit a more rapid means of distinguishing ASF from hog cholera than may be done with the present hemadsorption technique.

The epizootiological studies have shown that bush pigs, wart hogs, and porcupines may be inapparent carriers of ASFV.

The work under the P.L. 480 project in Spain is primarily on diagnostic studies. The hemadsorption test was applied to specimens from 243 suspected outbreaks of ASF. Of these, 172 were positive, 67 were negative, and 4 were toxic to the leukocyte cultures. In these investigations the hemadsorption effect was specific for African swine fever and bacterial contamination did not interfere with the test.

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# PARASITES AND PARASITIC DISEASES OF SWINE Animal Disease and Parasite Research Division, ARS

Problem. Parasitic diseases have been estimated to cost the swine industry of the United States at least \$200 million annually. These diseases for the most part are cosmopolitan. Subclinical infections are the most frequent type and the most costly, yet they are generally so difficult to recognize that they often are overlooked entirely. Diagnosis is difficult, and successful treatments for many of these parasitisms are not available. Moreover, management practices to avoid the spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling, or eradicating parasitic diseases so as to provide for healthy swine, insure adequate supplies of parasite-free pork for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a prosperous agriculture, a sound national economy, a high standard of living, and a healthy population.

#### USDA PROGRAM

The <u>Department</u> has a continuing long-term program involving parasitologists, veterinarians, biochemists, microbiologists, and pathologists engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The <u>Federal</u> scientific effort devoted to research in this area totals 4.7 professional man years. This effort is divided among sub-headings as follows:

The role of parasites in the economy of swine production 1.2 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and at the Division's laboratory at Tifton, Georgia, through informal cooperation with the Georgia Coastal Plain Experiment Station.

Bionomics and pathogenicity of the swine whipworm 0.5 at the Beltsville Parasitological Laboratory.

<u>Swine kidney worms</u> 2.1 at Tifton, Georgia, the Beltsville Parasitological Laboratory, and under cooperative agreement with the North Carolina Agricultural Experiment Station at Raleigh.

<u>Investigations of Trichinella spiralis</u> 0.5 at the Beltsville Parasitological Laboratory.

Effect of anthelmintic treatment on rate of gain 0.3 at Tifton, Georgia.

Pathogenic role of the intestinal roundworm O.l under a cooperative agreement with the Nebraska Agricultural Experiment Station at Lincoln.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 3.9 professional man years divided among sub-headings as follows: effect of anthelmintic treatment on rate of gain 0.4; swine kidney worms 0.5; pathogenic role of the intestinal roundworm 1.0; biology and pathology of the swine whipworm and other parasites 2.0. Work in the North Central Region deals with anthelmintic treatment, roundworms, and biology and pathology of the swine whipworm and other parasites. Work in the Southern Region deals with anthelmintic treatment, roundworms, and kidney worms. The Western Region is carrying out work on anthelmintic treatment.

Industry and other organizations. Several chemical companies are engaged in the formulation of compounds and explorations for chemicals that may be used safely in the treatment of swine for elimination of worms. Generally, these companies have their own facilities, including laboratories, barns, and other structures containing pens for experimental animals, and in some cases, pastures. The work of these companies and the results, expenditures, and related matters are ordinarily confidential, since they involve eventually saleable products. Estimated annual expenditures are equivalent to approximately 15 professional man years.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

# A. The role of parasites in the economy of swine production.

In 1961, at the Beltsville Parasitological Laboratory, studies of the lipid content of helminth parasites were initiated in an attempt to learn more about the fat metabolism of helminths. This type of investigation was only made possible by the development of new techniques and information obtained was useful in increasing our understanding of parasitic disease and of developing new methods for the control of parasites by chemical and biological methods. Four species of helminths and one species of lice were analyzed in this preliminary study and several higher carbon-number fatty acids were found to be present. The lipids in the helminths (swine nodular worms) were similar to those found in the intestinal mucus in which they live. The presence of odd-carbon-number fatty acids in these parasites indicated that the worms may feed on bacteria. The lipids of the louse were somewhat different from those found in the blood of the host. This finding indicated that the lice either metabolize the lipids or concentrate them.

One of the nodular worms of swine, <u>Oesophagostomum quadrispinulatum</u>, was found to consist of possibly three different strains. Two of these have a 20-day prepatent period, whereas the other has one of 50 days. One of the 20-day strains has tails shorter than the other 20-day strain. The tails of the latter and of the 50-day strain are relatively long. There is some evidence that the short-tailed form can be used for genetic studies.

The search for cheaper and more satisfactory materials than animal charcoal for use in making cultures of round worm eggs in swine feces resulted in the finding that peat moss, sphagnum moss, vermiculite, sawdust, sand, and topsoil all produce satisfactory results. Peat moss was considered the most satisfactory material for culturing eggs of the nodular worms and red stomach worms of swine. The relative quantities of water, feces, and the above-named materials and the thoroughness with which the ingredients were mixed were found to be quite important in obtaining large yields of infective larvae. The quantity of the various ingredients vary with the amount of moisture in the fecal sample. However, satisfactory results were obtained when 40 to 80 ml. of water, 20 grams of peat moss to 595 grams of sand were mixed with 100 grams of fecal material.

Studies indicated that adult nodular worms can become established in another swine host after being transferred to it by means of an enema tube via the anus. Seven pigs were successfully infected by this means.

Infective larvae of swine nodular worms were observed to be capable of infecting susceptible pigs after being stored at ordinary refrigerator temperatures ( $4^{\circ}C_{\bullet}$ ) for 575 days.

In 1962 further investigation of the lipids in the tissues of the swine nodular worm, Oesophagostomum quadrispinulatum, demonstrated that all five kinds of phospholipids were present - non-choline phospholipids, inositol phosphatide, lecithin, sphingomyelin, and lysolecithin. In addition, studies on two intestinal nematodes of chickens, Ascaridia galli and Heterakis gallinarum, the pork muscle worm, Trichinella spiralis, and one free-living nematode, Ditylenchus myceliophagus, disclosed the presence of 28, 33, 30, and 28 fatty acids, respectively. All of the last-mentioned compounds were straight chain fatty acids, whereas 10 of the 33 fatty acids reported from the swine nodular worm in 1961 had side chains. The three parasitic nematodes had a complete series of saturated straight chain fatty acids ranging from 9 to 20 carbon atoms. Fatty acids having 9, 13, 17, and 19 carbon atoms were missing from the free-living nematode. From 44 to 58 percent of the fatty acids in the parasitic worms were unsaturated, whereas 65 percent of those in the free-living nematode were in this group. The fatty acid having 16 carbon atoms and two double bonds was prevalent in the nematodes from the chicken. The most abundant fatty acids in the free-living nematode were those with 18 and 22 carbon atoms having one unsaturated bond. The significance of these findings is yet to be determined.

In 1962 at Tifton, Georgia, the effect of Strongyloides ransomi on the rate of gain of pigs of different ages and on different levels of nutrition was less than the effect of the age of the pigs and the levels of nutrition as compared with inoculated and non-inoculated, full-fed and limited-fed, and 80 lb. and 40 lb. groups. This was attributed mainly to the late establishment of infections in the inoculated groups of pigs.

Strongyloides ransomi infections in baby pigs may cause death or serious loss in thriftiness. Diagnosis of natural infections and observations of experimental inoculations given to pregnant sows indicate the possibility of prenatal infections of this species as a common occurrence.

Diagnosis of field cases of parasitism again showed <u>Strongyloides ransomi</u> to be the most prevalent parasite to cause death and unthriftiness in baby pigs. <u>Ostertagia ostertagi</u> was the most prevalent species in clinical cases of parasitism in cattle and was the cause of the loss of 6 brood cattle of 3 to 6 years of age.

# B. Bionomics and pathogenicity of the swine whipworm.

In 1961, at the Beltsville Parasitological Laboratory, observations on the period during which swine whipworm eggs, placed on the surface or buried in soil up to 8 inches, remain infective to pigs in the vicinity of Beltsville were continued. Eggs deposited during the summer months survived 4 and 1/2 years on the surface of the plots, and at depths of 4 and 8 inches. Eggs deposited during the winter months have survived 2 years on the surface of the soil and at a depth of 4 inches. However, samples from the surface of plots contaminated in June 1956, did not produce infections in pigs when administered to them in April 1961. The infectivity of the buried eggs was not tested at that time. These results demonstrated that the swine whipworm egg remains infective to pigs for as long as 4 and 1/2 years in the absence of recontamination, whether on the surface of the soil or buried as deep as 8 inches.

Several attempts were made to find and describe the early stages of the parasitic portion of the life cycle of the swine whipworm, which up to the present time have not been described. Pigs were fed 50,000 embryonated eggs of <u>Trichuris suis</u> and were necropsied on the 7th, 9th, 13th, 21st, and 23rd day after infection. In stained sections of the caecum and large intestine, portions of nematode larvae were found deeply embedded in the crypts of the mucosa on the 9th day, and on the surface of the mucosa on the 13th day. A few first-stage larvae were recovered from the lumen of the large intestine and cecum on the 23rd day, and the first- and second-stage larvae on the 21st day.

# C. Swine kidney worms.

In 1961, at Tifton, Georgia, the program for the eradication of kidney worms from an experimentally infected area at the Georgia Coastal Plains Experiment Station was completed. The program was based on limiting the breeding herd to young gilts and disposing of them after weaning their first litter. The incidence of kidney worm infections in pigs on contaminated lots was reduced from 88 percent (Spring, 1959) to 34 percent (Fall, 1959) to 0 percent in the Spring of 1960. A similar experiment is under way on a farm where a heavy natural infection of kidney worms was prevalent.

In 1962, at Tifton, Georgia, it was reported that the incidence of kidney worm infection in pigs on the experimental farm, raised from gilts in a kidney worm contaminated lot, was 93 percent for the spring group of 1961 pigs and 50 percent for the fall group. The incidence in pigs farrowed in an adjacent lot from gilts free of kidney worms was zero for both the spring and fall groups.

In 1961, in work performed under a cooperative agreement with the North Carolina Agricultural Experiment Station, Raleigh, there were indications that prenatal infection with swine kidney worms might occur if sows were exposed to kidney worm larvae during pregnancy. In pigs fed kidney worm larvae at 8 weeks of age, eosinophilia appeared at the second week and reached a peak at the seventh to ninth weeks, and continued at a high level through the sixteenth week. The sedimentation rate increased at the fifth week and continued at a high rate through the sixteenth week. A series of compounds, including the organic phosphate group, failed to stop ova production in kidney worm-infected sows when administered orally or by inoculation within a safe level of medication.

In 1962, at the North Carolina Station, in studies to substantiate prenatal infection, six gilts reared colostrum-free in air-lock isolation units, were given 500 infective S. dentatus larvae weekly. These doses began at first heat and will continue throughout gestation. The gilts were bred at second heat period. Arrangements have been made to obtain colostrum- and parasite-free pigs to be penned with the offspring of these gilts for positive controls. The pigs from the gilts will be weaned at 8 weeks of age and the sows autopsied to determine status of kidney worm infection in the dams. Blood samples will be taken from the pigs for cytological and immunophoretic studies. Postmortem examinations will be made of pigs from different litters at 6 and 12 months of age.

Sows were purchased from a local abattoir following demonstration of ova in urine. They were confined in a quonset hut with concrete floors that were scrubbed daily to remove source of reinfection. The ova in the urine of these sows were used as a source of infection for experimental animals. A large Duroc sow is passing viable ova in urine after three years. There was a slight drop in concentration of ova after 30 months. A Poland China sow is producing large numbers of ova after confinement for 16 months.

Several compounds have been evaluated as possible anthelmintics against the swine kidney worm. Naturally infected sows were purchased following the demonstration of ova in the urine. The compound under investigation was given orally or as an injection. Examinations were made of the urine to determine influence on ova production. Compounds evaluated included Bayer 13/59, Bayer 21/199, Bayer 29493, Ruelene, and Tennecetin. No satisfactory compound was found that decreased ova production within a safe level of medication.

Substantiation of prenatal infection would aid in the understanding of the life cycle of the kidney worm. This phenomenon could shorten the prepatent period by 2 to 3 months and help explain the presence of sexually mature parasites in young pigs. A patent infection in sows for as long as 3 years would greatly intensify the problem of control, as these sows could contaminate ground and provide source of infection for several litters of pigs.

### D. Investigations of Trichinella spiralis.

In 1961 at the Beltsville Parasitological Laboratory, it was reported that, although the standard digestion technique is considered to be a very dependable method for detecting trichinae in meat samples, it is time-consuming and requires relatively expensive and elaborate apparatus. A less complicated and more rapid method was therefore developed in which the psoas muscle of pigs infected with trichinae was blendorized, passed through a 100-mesh screen, and the sediment examined with a dissecting microscope. In this test trichinae were detected in a 20-gram sample of the psoas muscle of an 80-pound pig that had received 80 cysts of Trichinella spiralis. The digestion technique was then employed to determine the distribution of trichinae in 200-pound pigs that had received 10 to 100 cysts. Muscles of the neck, tongue, cheek, shoulder, loin, ham, diaphragm and chest wall, and the psoas muscle, were examined. More larvae per gram of tissue were usually found in the cheek, diaphragm, and tongue than in the other tissues, but no consistent pattern of distribution was noted.

In 1962 no work was reported for this project.

### E. Effect of anthelmintic treatment on rate of gain.

In 1961 and 1962, at Tifton, Georgia, the effect of anthelmintic treatment (sodium fluoride against Ascaris suum) on rate of gain when administered to parasitized pigs of different ages and on different levels of nutrition was less than the effect of the age of the pigs and the levels of nutrition. This was attributed mainly to the low rate of parasite infection.

### F. Pathogenic role of the intestinal roundworm.

In 1961, in research studies under a cooperative agreement with the Nebraska Agricultural Experiment Station, Lincoln, trypsin and chymotrypsin inhibitors were extracted from the body fluid and body wall of adult ascarids. Sixmonth-old pigs were as readily infected with ascarids as were five-week-old pigs. Liver lesions caused by migrating Ascaris healed within 21 days. Ascaris larvae migrated readily through the liver and lungs of pigs which had been given two previous exposures of infective Ascaris eggs. Thiabendazole, at 0.25 percent of a milk diet, greatly reduced the number of Ascaris larvae within the liver and lungs of 4 pigs. Ascaris infections have occurred in all of 30 herds of repopulated specific pathogen-free (SPF) pigs.

In 1962 at the Nebraska Station, subcutaneously injected thiabendazole effectively stopped migrating Ascaris suum. Hygromycin B, and a cadmium formulation effectively removed adult ascarids from swine. A N-Benyl derivative of Hygromycin was ineffective. A special formulation of an organic phosphate, 2,2 Dichlorovinyl dimethyl phosphate, was highly efficacious against A. suum and Trichuris suis in swine. Guinea pigs demonstrated a good immunity to A. suum when injected with infective eggs and specific protein components of the adult worms.

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## BEEF CATTLE, HORSE, AND SWINE INSECTS Entomology Research Division, ARS

Problem. Insects and ticks irritate and torment livestock throughout the year in all parts of the United States and cause serious These pests reduce weight gains, lower the quality of meat and hides, and spread numerous animal diseases. Livestock losses directly attributable to insects and ticks are estimated to exceed \$300 million annually. Practical but not adequate control methods have been developed for lice, screw-worms, ticks, bots, grubs, and other insects, but satisfactory methods of protecting cattle from horse flies, deer flies, stable flies, mosquitoes, and the newly introduced face fly remain an unsolved problem. The development of insecticides for use on beef cattle, horses, and swine has been hampered because certain insects have become resistant to various insecticides, and because harmful residues have been found in meat following the application of certain materials. Safe, effective, nonresidue-forming insecticides and repellents are required. Urgently needed are economical and long-lasting insecticides or repellents for range cattle to protect them against vicious biting flies. Safer, cheaper, and more effective systemic insecticides and more efficient means of administration are needed to combat grubs and bots in cattle and horses. New approaches to control, including radiation and chemosterilants, should be explored to determine their feasibility as practical control methods. Efforts also need to be made to find and evaluate insect pathogens, parasites, and predators for controlling certain livestock pests. Expanded basic studies on the biology and physiology of these pests are needed to find weak links in their life cycles that will serve as a basis for the development of more effective and safer methods of control. Research also is urgently needed on the role of insects in the spread of diseases of livestock.

### USDA PROGRAM

The Department has a continuing, long-term program involving basic and applied research on insects and ticks which affect the health and productivity of beef cattle, horses, and swine. Studies are conducted on the biology, physiology, genetics, and nutrition of the screw-worm, stable fly, horn fly, house fly, mosquitoes and other pests; on the nature of insect resistance to insecticides; and on absorption, metabolism and excretion of insecticides by insects feeding on or in animals; the effects of irradiation and chemosterilants on insects; insect attractants and repellents; and other new approaches to control. Research is concerned with the development of more effective contact and systemic insecticides and protective treatments for the

control of livestock pests. Studies are conducted to determine the occurrence of residues in tissues of animals treated with insecticides. Minor consideration is given to the development of sanitation and management procedures and biological control methods, including parasites and predators, for controlling the face fly, stable fly and several other pests. Emphasis is also given to the development of insect sterility, attractants and various other noninsecticidal approaches to control. Studies are conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions to evaluate various kinds of traps and devices for estimating and controlling natural insect populations, and improved or special equipment for the application of insecticides to animals. Limited research is conducted on the role of insects and ticks as vectors of livestock diseases, with particular emphasis on bovine anaplasmosis.

The Federal scientific effort devoted to research in this area totals 16.3 professional man-years. Of this number, 6.1 is devoted to basic biology, physiology and nutrition; 3.9 to insecticidal and sanitation control; 2.0 to insecticide residue determinations; 0.2 to biological control; 2.0 to insect sterility, attractants and other new approaches to control; 0.2 to the evaluation of equipment; 0.8 to insect vectors of diseases; and 1.1 to program leadership.

### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 11.1 professional man-years divided among subheadings as follows: Basic biology, physiology and nutrition 2.2; insecticidal and sanitation control 5.9; insecticide residues 0.7; biological control 0.8; insect sterility, attractants and other new approaches to control 0.6; evaluation of equipment 0.3; and insect vectors of diseases 0.6.

Industry, especially chemical companies, and other organizations are engaged in research on the formulation and evaluation of insecticides for the control of livestock pests. Industry also cooperates with Federal and State workers in developing information on residues resulting from the use of promising insecticides in connection with label registration. Estimated annual expenditures by industry are equivalent to approximately 10 professional man-years.

### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

- A. Basic Biology, Physiology and Nutrition
- 1. <u>Mosquitoes</u>. Studies on the biology of mosquitoes were conducted in Oregon, Nevada and California. At Reno, Nev., studies were continued

in cooperation with the Soil and Water Conservation Research Division on the biology and control of mosquitoes in relation to land and water management practices. In 1961 water supplies throughout the State were low; in March the reservoirs were nearly empty and water supplies ranged from about 15 to 50% of normal.

In Nevada further studies on the ranges of pH and salinity in the breeding areas of <u>Aedes niphadopsis</u> and <u>A. campestris</u> confirmed and slightly extended the ranges observed previously. No mosquito breeding was found in waters with total soluble salts as high as 67,200 p.p.m. Water of breeding sites for <u>Culex erythrothorax</u> ranged from pH 6.8 to 9.1; total soluble salts ranged from 237 to 7,320 p.p.m. Studies confirmed observations in 1960 that certain abandoned mines furnish excellent overwintering habitats for mosquitoes, especially <u>Culex tarsalis</u>.

Population emergence for Culex erythrothorax was 1 1/2 to 2 months earlier in southern Nevada than in northern and central areas of the State. The larvae overwinter in Nevada. In two breeding areas, heavy ice and destruction of emergent vegetation by migratory ducks and geese reduced the larval population noted the previous November by about 97%. In other areas, a gradual build-up of pupae was observed in April and pupae predominated in late April and early May. A colony of this species had been established in the laboratory. Aedes campestris, believed to be a univoltine species (one brood per year) produced a large brood in June following heavy rainfall. Normally this species breeds only in the spring. Biting adults of Anopheles freeborni were noted in early February in southern Nevada; some Culex tarsalis activity occurred in late March in the valleys. Only a few Aedes niphadopsis could be induced to feed on humans although this species has been reported a vicious biter of livestock and humans. Breeding of Aedes increpitus began as early as February 10 at an elevation of 4,500 feet; at 6,300 to 6,700 feet breeding began in mid- to late March. In the spring a survey of 74 suspected mosquito breeding sites showed Aedes niphadopsis to be the dominant species in 65% of the sites, whereas Aedes campestris was more abundant in 28% of the sites. Aedes dorsalis predominated in only 7% of the samples. Ten species of mosquitoes were autogenic (capable of oviposition without a blood meal): Aedes campestris, A. communis, A. dorsalis, A. melanimon, A. niphadopsis, A. nigromaculis, A. schizopinax, Culex erythrothorax, C. tarsalis, and Culiseta incidens. Only Aedes communis and Culex tarsalis had previously been reported autogenic.

Surveys were continued on biting arthropods of the Humboldt River Basin of Nevada in connection with a major study of water resources being carried out cooperatively between the U. S. Departments of Agriculture and Interior, the University of Nevada, and the Nevada State Department of Conservation and Natural Resources. For the third successive year, waterflow was only about one-fourth of normal and very few breeding areas received water. Only light breeding of Aedes melanimon, A. vexans, Culex tarsalis, and Culiseta inornata was observed. Very few biting adult mosquitoes were noted. Tabanids (horse and deer flies) and black flies were also negligible.

At Fresno, Calif., research on control of mosquitoes in relation to irrigation practices was conducted in cooperation with the California Bureau of Vector Control. Studies showed that dairy drains are sites of heavy breeding of <u>Culex quinquefasciatus</u> through November and into early December. The breeding in such locations probably contributes heavily to the overwintering adult population of this species. Autogeny (ability to lay eggs without a prior blood meal) was observed in Culiseta inornata and <u>C. incidens</u> but not in other species.

At Corvallis, Ore., studies on flight movements of <u>Culex tarsalis</u> indicated they move from their resting stations about sunset and return about sunrise. The instinct of <u>tarsalis</u> to oviposit in low sites is stronger than the instinct for oviposition in favorable waters. Female <u>tarsalis</u> mate only once whereas males mate several times. Laboratory studies indicated that 4th instar <u>Aedes sierrensis</u> larvae enter diapause from about the last week in <u>September through the third week in December</u>; diapause can be induced in the laboratory through manipulations of the photoperiod and broken by constantly increasing day lengths.

Studies were continued in Oregon on the status of insecticide resistance in mosquitoes. Tests showed that DDT-resistant Culex tarsalis larvae breeding on irrigated farms and in log ponds were also resistant to closely related compounds, but not to dieldrin or lindane. Further spread of resistance in Culex mosquitoes was indicated when Culex peus showed some apparent resistance to DDT and tarsalis, for the first time, showed some resistance to malathion. Studies on the physiology of resistance showed malathion-resistant larvae of C. tarsalis to be more efficient in regulating salt (chloride) uptake than susceptible larvae during exposure to malathion. Resistant and susceptible larvae take up similar amounts of chloride when exposed to 1% sodium chloride alone. Exposure to 1% sodium chloride resulted in an increase of oxygen consumption in susceptible and malathion-resistant larvae, but chloride had no measurable effect on cholinesterase inhibition or accumulation of malaoxon, the principal breakdown product Studies of the insect enzyme systems of malathion in mosquitoes. cholinesterase and aliesterase, led to the development of synergists for organophosphorus insecticides. Several tris-substituted derivatives of phosphoric acid overcame the resistance of tarsalis larvae to malathion, increasing its toxicity 100-fold to a resistant strain.

2. Stable Fly. Studies were continued at Lincoln, Neb., on the biology of stable flies. Immature stages of the stable fly overwinter in protected situations. None survived in environments subject to frequent freezing and thawing. The first adult flies appeared on June 1 and gradually increased in numbers through June. Peak populations occurred during July and August.

Efforts were continued in Nebraska to induce resistance to DDT in stable flies. After selection with topical applications of 0.005-0.075% DDT for 34 generations, no resistance was apparent and the colony was discontinued. However, when 36 p.p.m. of DDT was placed in the larval medium, 3-fold resistance was indicated in 6 generations. After 11 generations (1 year), the colony tolerated 500 p.p.m. in the media and some larvae survived 625 p.p.m. Resistance was about 14-fold.

3. Face Fly. In Nebraska, adults of the face fly hibernating in homes became active in February. Collected females contained well developed eggs but did not oviposit in the laboratory. Adults were first observed on cattle at Lincoln on May 14 and by late June herds were lightly infested. High populations persisted from early July until cool weather.

After considerable investigation of nutritional requirements, a thriving colony of the face fly was developed under laboratory conditions. The adult diet consisted of cattle feces, citrated blood, milk, dimalt and bee pollen. Larvae were reared in cattle feces and pupated in a mixture of sand and vermiculite. The life cycle (egg to egg) was 14 days at 82° F. and 28 days at 70°.

4. Horn Fly. Studies on the biology of the horn fly were continued in Texas and Oregon. In Texas intensive spraying was employed to eradicate horn flies at one location during the late fall, whereas flies persisted in other locations until early December. In the spring flies reappeared at untreated locations nearly 4 weeks earlier than at the treated location. The flies overwintered as diapausing pupae and prepupae. In Oregon, the horn fly overwintered as pupae in cattle droppings but house flies and stable flies failed to survive. Adult horn flies began emerging from overwintering pupae on May 17 and emergence was complete by June 24. Emergence ranged from 0.8 to 14.8% in exposed droppings and from 3 to 61% in those protected by screen cages.

In laboratory studies in Oregon occasional eggs of the horn fly hatched at a constant temperature of  $52^{\circ}$  F. and the larvae developed to pupae, but no adults emerged from them. Adults also did not emerge from

1-day old pupae reared at  $80^{\circ}$  F. and then kept at a constant temperature of  $52^{\circ}$  for 3 months but when returned to  $80^{\circ}$ ,20% produced adults in 5 days. When 5-day-old pupae were transferred from  $80^{\circ}$  to  $52^{\circ}$  F., adult emergence occurred between 7 and 23 days, whereas at  $80^{\circ}$  it was complete in 1 to 3 days.

In Texas a laboratory colony of the horn fly has been developed and maintained for 10 generations without contact with a bovine host. The adult diet consists of bovine blood, ground beef juice and an antibiotic to prevent spoilage. The optimum temperature for survival and reproduction is 90° F. Larvae are reared in feces of cattle fed alfalfa hay. The feces of cattle fed sorghum or prairie grass proved unsatisfactory as larval media.

5. Screw-worm. Investigations on the screw-worm were conducted at Kerrville, Tex. The sexual development and behavior and genetics of the screw-worm fly were studied. Dissections showed that egg development was synchronous in all ovarioles. The exact stage of development of the oocyte and the nurse cells and the cytological condition of the nucleus were determined for various ages of females. Males began mating when 2 days old but were not vigorous in mating until 4 days of age.

The number of adults in holding cages and mating activity had pronounced effects on longevity. Optimum longevity occurred when not over 100 adults were confined in 12 x 12 x 20-inch cages. Adults also lived longer when the sexes were kept separate and when the male to female ratio was reduced. Excess mating by males and harassment of females by males reduced longevity.

Efforts were made to develop a genetically distinct strain of screwworm fly. In examining many thousands of adults from scores of strains, a black mutant male was discovered. Selected matings resulted in the development of a homozygous black strain. When this strain proved inferior to normal strains in longevity and mating vigor, new genes were introduced by mating black females to normal blue males. After inbreeding for several generations, the black strain was equal to normal strains in longevity and mating vigor. Several additional new strains have been developed from larvae and adults, including one in which the scutellum is fused laterally to the scutum. Linkage studies with "black" and "interrupted" marker strains showed that these genes are sex-linked on the "X" chromosome. Crosses of these strains did not produce the expected number of black males, and females homozygous for the "interrupted" gene did not survive as well as the heterozygotes.

Efforts to formulate a completely synthetic larval rearing medium have proved unsuccessful so far, but progress has been made in developing a modification of the standard ground meat-blood medium. Screwworm larvae reared for 48 hours on the standard medium consisting of 50% horse meat, 30% bovine plasma and 20% water, were successfully transferred and completed development in media in which 50, 75, 87.5 and 100% of the horse meat had been replaced with fish flour. The substitution of the fish flour reduced production costs to as low as 8.7¢ per thousand pupae, compared with 21.4¢ for the standard medium. Weights of the larvae ranged from 73.0 to 69.6 mg. for the 50 to 87.5% substitutions, as compared with 74.6 mg. for the standard. The 100% substitutions yielded larvae that weighed only 60.2 mg., but the adults lived slightly longer than those produced on the standard medium. In cage tests the small males and normal-sized males mated about equally as well with normal-sized females.

Intensive studies were made on the effects of irradiation on the sexual development, mating ability and longevity of the screw-worm. A dosage of 5,000 r completely sterilized both sexes. position resulted from matings of irradiated males and females and normal males and irradiated females, wereas matings of irradiated males and normal females resulted in normal oviposition but none of the eggs hatched. Female flies 0 to 0.4 hours old were more sensitive to irradiation than 5-day old pupae. A dosage of 2,000 r reduced ovarian growth by 50% in young females but had no significant effect on ovarian growth in females irradiated as 5-day old pupae. Irradiation had little or no effect on ovarian growth in females over 48 hours old. However, oocytes in 4- to 5-day old females were damaged more by irradiation than those in 3-day old flies. No difference was noted in the longevity of 5-, 6-, and 7-day old flies irradiated with 7,500 r in one, two, or three exposures. The number of dominant lethals and longevity of adults were unchanged regardless of whether 5-day old pupae were irradiated with a single dose or equal fractions at intervals of 8 and 24 hours.

There was no consistent difference in the effects of 1,000 and 5,000 r administered as a single dose or two equal doses. Female flies 3, 4, and 5 days old differed greatly in radiation sensitivity but dominant lethals were induced in the oocytes of all ages. In additional tests with 5-day old pupae, which have only oogonial cells, the number of eggs deposited by females decreased as the radiation dose increased, indicating that damage to some cells eliminated them from the germ line and that they were not rapidly replaced.

Additional studies on the effects of irradiation on 4-, 5-, 6- and 7-day old screw-worm pupae showed decreased longevity of adults with increased dosage. All stages showed lower mortality at 2,800 r than at higher dosages.

When female screw-worms were irradiated as 5-day old pupae in a well-aerated container, a dose of 5,000 r produced 99% sterility. When the same dose was given in an atmosphere of carbon dioxide, less than 50% sterility resulted. However, irradiation in an atmosphere of 50% air and 50% CO2 produced a greater effect than in air alone. These results suggest that the irradiation capacity of the present Cobalt-60 sources could be increased by 38% by irradiating with 5,000 r in 50% air-50% CO2. For this synergistic effect to be shown, it is necessary that the insects be held in the CO2-air mixture for at least 30 minutes prior to, as well as during, irradiation. It should be a simple engineering job to modify the currently used procedures for such irradiation.

Studies were conducted on the absorption, distribution, metabolism, and excretion of P<sup>32</sup> metepa (chemosterilant). Metepa was absorbed faster and excreted more slowly by the stable fly than the screwworm fly. This may account for the fact that the sterilizing dose of metepa is much less for the stable fly than for the screw-worm fly.

Field cage studies showed that mature screw-worm larvae released on soil at 73° F. entered the ground in 3-4 minutes and pupated in 24 hours. Peak emergence of adults occurred 12 days later between 5:00 and 8:00 a.m. Adults did not feed or exhibit mating activity until 4 days old. Adult survival was high for 9 days but very few adults were alive after 22 days. In similar releases when soil temperatures were 51° F., few larvae entered the soil and although many were alive after 1 week, none had pupated.

Work was conducted on the dispersal and behavior of marked released adult screw-worm flies. The first night after release from 0.4 to 10% of the marked flies were recovered within 25 yards of the release point. Recoveries decreased on successive nights. In some tests none apparently moved over 400 yards, whereas in others, dispersion was rapid, as far as I mile in 45 minutes. Over 90% of the adults were found resting near the tips of leafless twigs within 4-5 feet of the ground.

6. House Fly. The following report on these insects covers work conducted at Corvallis, Ore.

DDT-resistance in house flies was attributable to the ability of the flies to dehydrochlorinate the insecticide. The mechanism of resistance in house flies to the carbamates was due to aliesterase activity, the same as for the organophosphates.

 $P^{32}$  memepa (chemosterilant) was rapidly absorbed and metabolized by house flies and mice. Almost complete degradation occurred in  $2^{l_4}$  hours with phosphoric acid being the major metabolic product.

Irradiation of resistant flies with 1,000 r did not alter their susceptibility to insecticides or esterase activity. Radiated females mated with normal males produced normal numbers of eggs but only 45% were fertile.

Further studies were conducted with colonies of normal and parathion-resistant flies that had been irradiated as pupae with 600 r for 7 generations. Only about 25% of the eggs hatched from matings of irradiated males and females of either colony. No changes in either esterase activity or insecticide susceptibility were apparent.

Certain synergists greatly increased the toxicity of malathion to resistant strains of house flies. The synergists, simple tris-substituted derivatives of phosphoric acid, completely overcame high levels of resistance when used at 1:1 or higher ratios of synergist to insecticide. The most effective materials increased the toxicity of malathion from 36- to 40-fold against resistant house flies. Only about a 2-fold increase was indicated against susceptible strains of house flies. The relative ability of several of these materials to synergize malathion against resistant house flies was directly related to the inhibitory effect of the synergists on aliesterase activity. Strong synergists for malathion inhibited aliesterase activity at concentrations as low as 10<sup>-5</sup> M, while related nonsynergistic materials failed to inhibit the ali-esterase at concentrations up to 10<sup>-2</sup> M. The synergists may actually inhibit the mutant aliesterase present in all organophosphateresistant fly strains.

House flies treated with the synergist tributyl phosphorotrithioate and then treated with either parathion or paraoxon accumulated greater quantities of paraoxon than did flies treated with the toxicants only. This was true with both susceptible and parathion-resistant strains of flies. Measurements of the inhibition of thoracic cholinesterase activity provided a far better picture of the toxic action of organophosphates than did measurements of head cholinesterase.

Selection of a house fly colony with Isolan produced a strain with 3-fold resistance in 14 generations. At the same time, levels of esterase activity to methyl butyrate declined to 40% of the original level in flies of the selected strain. This same phenomenon occurs when house flies are selected with organophosphates, indicating that the same mechanism is responsible for resistance to both classes of insecticides.

House flies were capable of dispersing a distance of at least 5 miles in 24-48 hours.

7. <u>Cattle Grubs</u>. Research was initiated in Texas and Oregon to determine the nutrition requirements of cattle grub larvae and to develop

an artificial medium for rearing larvae under laboratory conditions. Second and third stage larvae have been successfully reared to maturity in serum in shell vials, but adult emergence from the pupae was quite low. Efforts to develop a suitable medium and techniques for rearing first stage larvae have proved unsuccessful so far.

- 8. Horse Flies and Deer Flies. Studies were continued in Mississippi on the biology of tabanids. The earliest species, Tabanus lasiophthalmus, appeared on March 31, reached a peak by late April, and disappeared by late May. T. vittiger schwardti, the most prevalent species, appeared April 5, was abundant from mid-May until early July and small numbers persisted until cool weather. T. fuscicostatus appeared May 11, reached a peak in June and disappeared by early September. Other species of lesser importance and dates of their occurrence were as follows:
  T. proximus, June 8 to September 7; T. lineola, April 26 to Oct. 5;
  T. americanus, May 24 to August 17; and Chrysops flavida, May 11 to Oct. 19. A total of 18 species was collected during the season.
  Several species of adult flies were collected and fed in confinement on cattle. Females of T. proximus and T. vittiger schwardti produced egg masses in captivity and the eggs hatched in 7 and 4 days, respectively.
- 9. Lice. Studies were conducted in Oregon to determine the factors involved in the decrease of cattle louse infestations during the spring and summer and the increase of populations during the fall and winter. The free cattle harbored very few lice after shedding their winter coat in the spring, but when they were restrained heavy infestations developed within a month. When released the cattle licked and rubbed themselves constantly for several days, causing a precipitious decline in louse populations. During the winter the heavy coats on the animals apparently minimized the effects of rubbing and licking and as a result high louse populations persisted throughout the winter.
- 10. <u>Ticks</u>. Work in Texas indicated that irradiation at the rate of 1,000 r had no effect on the molting of unfed or engorged nymphal lone star ticks. However, a dose of 2,500 r prevented molting of unfed and 1-day engorged ticks and only 3% of those engorged 1 week molted to adults. Exposure of newly molted nymphs to 1,000 r completely sterilized the males but not the females.

# B. Insecticidal and Sanitation Control

1. <u>Mosquitoes</u>. In Oregon studies were continued to find repellents for protecting animals from attacks by mosquitoes. None of 200 compounds screened in spot tests on cattle showed promise. Other

screening tests were conducted with a number of new insecticides against larvae and adults of <u>Culex tarsalis</u>. Three of the materials gave 100% kills of larvae at a concentration of only 0.025 p.p.m. In spray tests seven materials were 2 to 9 times more toxic to adult mosquitoes than malathion (standard) and two of these compared favorably with parathion.

2. Stable Fly. In Texas 447 compounds were screened in spot tests on cattle for repellency and toxicity against the stable fly. Of these materials 9 were Class IV repellents at 5%, 8 were Class IV repellents at 10%, 3 were Class IV toxicants at 0.5% and 2 were Class IV toxicants at 5%. The outstanding repellents were diamyl tartrate, 3-ethyl 2,4-octanediol, benzyl acetoacetate, piperonyl chrysanthemumate and Hercules AC-6432. Three other materials were slightly less effective. The most effective toxicants were Geigy G-27365 and Shell SD-2359.

Comparisons were made of the toxicities of various insecticides to stable flies. The LD-50's in µg/fly for the materials tested were as follows: Ronnel, 0.015; methoxychlor, 0.057; DDT, 0.072; DDT and WARF, 0.091; toxaphene, 0.17; diazinon, 0.021; pyrethrins, 0.021 to 0.028; and pyrethrins and piperonyl butoxide, 0.017.

Large cage tests were conducted on calves to compare the residual effectiveness of a number of new insecticides and methoxychlor (standard). Ruelene, Baytex, Bayer 22408 and Sevin were effective for 3 to 5 days against stable flies but were less effective than methoxychlor.

Extensive tests with pyrethrum showed that ultra-violet light was the most important factor in its rapid loss of effectiveness, although infrared was also deleterious. Wave lengths in the range of 2600-4000A caused the greatest loss in effectiveness of pyrethrum. Several hydroquinone antioxidants and lanolin prolonged the toxicity of pyrethrum about two-fold under irradiation. Several phosphoric acid derivatives increased the effectiveness of malathion against stable flies but had no effect on Sevin or methoxychlor.

Of 111 compounds tested as systemics in Texas, only 8 caused mortality of stable flies feeding on guinea pigs. Hercules 7522-H and Rhodia RP-9895 were effective orally at 50 and 100 mg./kg. The other six materials were effective orally at doses of 10 to 50 mg./kg. but their chemical names are confidential. Thirty Class IV repellents were administered orally to guinea pigs to determine if they would prevent feeding by stable flies. All were ineffective.

3. Face Fly. In Nebraska, a number of insecticides were administered to cattle in feed in order to determine levels necessary to prevent fly

breeding in the feces. Daily doses of V-C 13 at 4 mg./kg., Cygon at 5 mg./kg. and Co-ral at 10 mg./kg. gave 100% control of breeding but seven other materials were only partially effective at the doses tested. Sprays of methoxychlor, malathion, Delnav and synergized pyrethrins provided little or no control of this pest. Halters treated with DDVP, Dipterex and methoxychlor reduced fly populations but did not provide satisfactory control. Effective control of breeding was obtained by feeding Co-ral at 0.5 mg./kg. and ronnel at 7.8 mg./kg. daily but adult populations on the cattle remained high, presumably because of migration from adjacent farms. Weekly applications of 0.08 lb/acre of Co-ral on pastures prevented fly breeding in droppings of cattle but here again no effect on adult populations was apparent.

Horn Fly. In Texas tests were conducted on calves in large cages to compare the residual effectiveness of a number of new insecticides and methoxychlor (standard). Ruelene, Baytex, Bayer 22408 and Sevin were effective for 7-10 days as compared with 17 days for methoxychlor. Extensive field tests were conducted in Texas and Mississippi to compare several old and promising new insecticides for the control of horn flies on cattle. In Texas effective control was obtained with 0.25% sprays of GC-4072 for 15-21 days, with 0.06% diazinon for 8-12 days, and with 0.5% Dilan, Dipterex and methoxychlor for about 2 weeks. In Mississippi control was obtained for 11-14 days with 0.1 to 0.25% sprays of Shell 4294; for 7-10 days with 0.25% Sevin and with 0.1% Baytex; for 3 weeks with 0.5% Dilan and methoxychlor; and for 10-30 days with 0.25 to 0.5% Shell 4294. In season-long tests, effective control of horn flies was maintained with 4-5 sprayings of 0.25% Shell 4294, with seven sprayings of 0.1%, and with two applications of 2% in oil to backrubbers. Effective control was also maintained with mist sprays of 1% Shell 4294 applied weekly and of 0.05% pyrethrins and 0.01% DDVP applied daily.

Extensive tests were conducted to determine the effectiveness of low level feeding of insecticides in controlling horn flies. In Texas daily dosages of 7.8 mg./kg. of ronnel prevented horn fly breeding in droppings and reduced adult populations to a sub-annoying level within a week. Free choice feeding by cattle on ronnel salt blocks also provided very good control. Other effective materials and dosages were as follows: Co-ral, 0.5 mg./kg.; Bayer 22408, 1 mg./kg.; GC-4072, Baytex, Bayer 34727 and Stauffer R-1504, 2.5 mg./kg.; and Bayer 37342, American Cyanamid 38023 and Bayer 37341, 10 mg./kg. In Mississippi free-choice feeding on ronnel salt blocks and daily feeding at 4 mg./kg. prevented breeding in droppings and adult fly populations decreased significantly.

5. <u>Screw-worm</u>. Research was continued in Texas to develop more effective insecticides for controlling screw-worms affecting live-stock. One hundred thirty-one new compounds were screened for

systemic action by administering them orally and subcutaneously (SC) at several dosages to guinea pigs artifically infested with screwworms. Ten of the materials showed systemic action in one or both types of administration. The active compounds and minimum effective dosages in mg./kg. were as follows: Stauffer N-2310, Stauffer N-2599 and Stauffer N-3055, 25 mg. orally and 50 mg. SC; Stauffer N-3054, 10 mg. orally and 50 mg. SC; Hercules 9699, 50 mg. orally and SC; Stauffer R-3422, 50 mg. orally and 100 mg. SC; Rhodia 9895, 50 mg. orally; and Hercules 7522H, 100 mg. orally.

Screw-worm larvae surviving the screening tests of new compounds were collected and reared to adults. The adults were mated and records made of the number of eggs and percent hatch to determine if the chemicals screened produced any sterilizing effects. Of 121 compounds used in this study 13 adversely affected survival and development of larvae and pupae or oviposition and egg hatch. The most active materials and their effects were as follows: Bayer 38636 at 5 mg. orally, all male survivors; Shell 7079 at 50 mg. orally, no oviposition; Stauffer R-2404 at 10 mg. orally and SC, no hatch; ENT 5734 at 100 mg. SC, adults emerged but died without ovipositing; and Dilan at 500 mg. orally, no adult emergence from pupae.

6. <u>House Fly</u>. Extensive research was conducted at Orlando, Florida, on the control of the house fly in dairy barns, poultry houses, households and industrial establishments. Since the results of this research in Florida are applicable to the control of house flies affecting beef cattle, horse and swine, a brief resume of recent progress follows:

One hundred ninety-five new compounds were screened for residual effectiveness against house flies. Twenty-four materials were 90 to 100% effective for at least 24 weeks as compared with 12-16 weeks for malathion (standard). These materials included Thiodan, General Chemical GC-3583, Shell SD-4402, Upjohn TUC U-12927 (with synergist); Bayer compounds 29952, 30237, 30468 and 29492; Hooker HRS-1422, Geigy G-27365, Stauffer N-2404, heptachlor epoxide and twelve confidential compounds. Bayer 29952, Bayer 30237 and seven of the confidential compounds were still 100% effective after 44-48 weeks and are considered unusually promising for controlling flies. Several promising new compounds were evaluated as space sprays against susceptible and resistant strains of house flies. Some of the compounds were superior to malathion (standard) against susceptible flies but only one, Bayer 30911, was superior against resistant strains.

Over 50 selected compounds were evaluated as bait toxicants against resistant and normal strains of house flies. Eight of the compounds were equal or superior to Dipterex (standard) against both strains

of flies, namely: Bayer 30237, Bayer 30554, General Chemical 6506, Stauffer N-2230, Stauffer N-2404, dimethoate and two confidential compounds. All of the compounds tested were more effective against normal than against resistant flies.

Several materials which increased the effectiveness of malathion in laboratory tests were evaluated as residual treatments in barns against large populations of flies. Combinations of several synergists and malathion alone were equally ineffective in these tests.

7. Cattle Grubs and Other Bots. Research was continued in Texas, Oregon, and Nebraska to develop more effective insecticides for controlling cattle grubs and other bots affecting livestock. In Texas 131 new compounds were screened for systemic action by administering them orally and subcutaneously (SC) at several dosages to guinea pigs artifically infested with screw-worms. Ten of the materials showed systemic action in one or both types of administration. The active compounds and minimum effective dosages in mg./kg. were as follows: Stauffer N-2310, Stauffer N-2599, and Stauffer N-3055, 25 mg. orally and SC; Stauffer N-3047, 25 mg. orally; Zectran, 25 mg. orally and 50 mg. SC; Hercules 9699, 50 mg. orally and SC; Stauffer R-3422, 50 mg. orally and 100 mg. SC; Rhodia 9895, 50 mg. orally; and Hercules 7522H, 100 mg. orally.

In Texas further tests were conducted on small numbers of cattle with a number of compounds that had shown promise in screening tests or on individual cattle in 1960 and 1961 and with older effective materials administered in different ways. Ten of the new materials gave 98-100% control of grubs by one or more routes of administration. These materials, the effective doses, and routes of administration were as follows: Famophos, 25 mg./kg. intramuscularly (IM) and 10 mg./kg. in feed for 10 days; Bayer 37341, 0.5% spray, 2% pour-on, 10 mg./kg. orally, and 5 mg./kg. in feed for 10 days; Bayer 34727, 0.5% spray and 25 mg.kg. orally; Stauffer 3352, 25 mg./kg. orally; Stauffer 3828, 100 mg./kg. orally; Stauffer R-1504, 0.5% spray, 2% pour-on, 50 mg./kg. orally and 25 mg./kg. in feed for 10 days; Bayer 37342, 0.5% spray, 25 mg./kg. orally, 15 mg./kg. IM, 5 mg./kg. in feed for 10 days and 10 mg./kg. in feed for 6 days; Bayer 37289, 25 mg./kg. orally; Bayer 42,600, 25 mg./kg. orally; and Rhodia, 100 mg./kg. orally.

The older materials, dosages and routes of administration giving 98-100% control of grubs were as follows: Co-ral, 2% and 8% pour-on; Dipterex, 5 mg./kg. in feed for 10 days, 6% pour-on and 150 mg. orally; Baytex, 2.5 mg./kg. in feed for 10 days and 10 mg. IM; Butonate, 5 mg. in feed for 5 days and 10 mg. in feed for 10 days; and Ruelene, 10 mg. IM and SC. In additional tests with GC-4072 one and two sprays of 0.25% gave 94% control. Several other materials were 78-91% effective by one or more methods of administration.

Extensive field tests with government and cooperator herds of cattle were conducted in Texas, Oregon and Nebraska to evaluate the effectiveness of promising new and several older systemics at different rates and various methods of administration. In Texas 99-100% control of grubs was obtained with Co-ral at 5 mg./kg. IM and as a 4% oil solution applied to the backline by the pour-on method. Pour-on applications of 4% in oil and 2% in oil and water and 0.5% sprays were over 90% effective. Dipterex applied as a 1.5% spray and at 4% and 7.75% pour-on treatments gave 99-100% control of grubs. pour-on applications of ronnel were 99% effective. Ruelene gave 95-100% control when applied at 2%, 4% and 7.75% by the pour-on method and as a 0.5% spray or dip. Baytex as a 1% pour-on and as a 0.25% spray gave 96 and 98% control. New materials giving 94-99% control were Bayer 37342 as a 0.5% spray and Famophos at 15 mg./kg. IM. of the older and newer materials failed to provide satisfactory control at lower dosages or by other methods of administration.

In Texas tests involving a number of cooperator herds were conducted to evaluate ronnel in salt blocks and as a feed supplement for the control of grubs. Feeding ronnel at the rate of 7.8 mg. daily for 14 days failed to give satisfactory control. Consumption of 2.6 to 2.8 mg./kg. daily of ronnel from the salt blocks for 4 months gave 78 to 86% control of grubs. Excellent control of 97% was indicated in herds given 7.8 mg./kg. of ronnel for 14 days and then provided with ronnel salt blocks for 3 months.

In Nebraska field tests were run to evaluate the effectiveness of Famophos as an intramuscular (IM) injection against cattle grubs. Injections of 15 mg./kg. gave 99% control as compared with 90% for 7.5 mg./kg.

In tests in Oregon with older materials, 98-100% control of grubs was obtained with the following treatments: Ruelene as 0.25 and 0.5% sprays; Dipterex as 1% and 2% sprays; Co-ral as a 0.5% spray; Baytex as a 0.25% spray, and a 25 mg./kg. in oil pour-on treatment, and in 5-day feeding tests at 5 mg./kg. Baytex also gave good but variable control at lower rates as sprays and pour-on treatments. In tests against grubs already present in the backs of cattle 85-100% kill was obtained with pour-on treatments of Baytex at 25 mg./kg. and with 2.5% dusts of DDVP and Dibrom. In tests with new materials excellent control was obtained with Bayer 37342 as a 0.25% spray and orally at 50 mg./kg. and with Famophos as a pour-on at 15 mg./kg. and in feed at 10 mg./kg. for 5 days. Bayer 37341 was highly effective as a 0.25% spray in some tests but not in others. Lower dosages of these materials and all dosages of several other new materials failed to give satisfactory control of grubs. Feeding of ronnel at the rate of 7.8 mg./kg. daily for 14 days resulted in reductions of 94-98% in grubs in several groups of cattle.

In Oregon topical application tests showed that male  $\underline{H}$ . bovis adults were more tolerant of ronnel than females. The LD-100's were 180 and 100  $\mu$ g/fly, respectively.

In Texas and Mississippi several of the better cattle grub systemics were evaluated against two species of horse bots. The most effective treatments were Butonate and Ruelene at 50 mg./kg. in feed; Famophos at 50 mg./kg. by stomach tube; Bayer 37341 at 50 mg./kg. by stomach tube and in feed. Tests in Mississippi indicated that dosages of 30 to 50 mg./kg. of Ruelene in feed were highly effective against horse bots but lower dosages did not give satisfactory control.

- 8. Horse Flies and Deer Flies. In Mississippi daily applications of synergized pyrethrins with an automatic sprayer greatly reduced the number of horse flies and deer flies attacking cattle. Sprays of insecticides, such as toxaphene, malathion and Ciodrin, were ineffective in repelling biting flies but a good percentage of those that engarged died subsequently from contact with the insecticide.
- 9. Lice. In Mississippi, twenty-five promising new insecticides were evaluated in spot tests in comparison with methoxychlor against cattle lice. Two materials, General Chemical 4072 and Bayer 37342, prevented reinfestation for 12 days as compared with 7 days for methoxychlor. Heavy infestations of horse lice were eradicated with 0.5% malathion sprays. Nine materials were evaluated for systemic action by giving them orally to louse-infested cattle. Co-ral caused 100% kill of all motile lice but the other materials were partially or completely ineffective at the dosages tested.

In Texas extensive field tests were run to compare the effectiveness of several of the newer insecticides against cattle lice. Sprays of 0.25% GC-4072 eradicated lice in a majority of tests but in others, light infestations developed within 2-4 weeks. Ronnel and Shell 4294 at 0.25% gave 100% immediate control but light reinfestations were apparent in all locations in 2-4 weeks. Ruelene applied at the rate of 75 mg./kg. along the backline eliminated louse infestations but lower dosages of 25 and 50 mg./kg. were not completely effective. Feeding of 50-75 mg./kg. of Ruelene over a period of 3 days reduced louse infestations only 40-50%. Daily feeding of Co-ral at 0.25 mg./kg. and Bayer 22408 at 0.5 mg./kg. for 3 weeks or more had no noticeable effect on louse infestations.

In Oregon complete control of cattle lice was obtained with sprays of 0.5% Co-ral, 0.25% Baytex and 0.75% lindane. These treatments also gave complete control of cattle scabies (Chorioptes bovis).

10. Ticks. Work on ticks was confined to the Texas laboratory. Only 4 of 131 compounds screened for systemic effectiveness showed systemic action against ticks engorging on treated guinea pigs. The effective materials, dosages (mg./kg.), and routes of administration were as follows: Rhodia, 25 mg. orally and 50 SC; Hercules 9699, 50 mg. orally and SC; Stauffer N-2310, 50 SC; and Zectran, 50 mg. orally.

Evaluation was made of the effectiveness of 71 insecticides against Boophilus ticks, using the dipping technique. A majority of the materials were toxic to this species, but the most effective were lindane, Co-ral, General Chemical 3582, General Chemical 4072, Bayer 25141, Bayer 29952, Bayer 30237, and Bayer 37341.

Extensive field tests were conducted to compare the effectiveness of several insecticides against the winter, lone star and black-legged ticks on cattle. Complete control of existing infestations of the winter and black-legged tick was obtained with sprays of 0.1% General Chemical 3582, 0.25% Shell SD-4294 and General Chemical 4072, and 0.5% methyl Baytex and toxaphene. Dilan at 0.5% and V-C 13 at 0.25% eliminated the black-legged but not the winter tick. Sprays of 0.05% diazinon and 0.5% V-C 13 gave lower immediate kills of the winter tick than 0.5% toxaphene but all three treatments were 99-100% effective after 1 week. In tests on horses, 0.25% sprays of GC-4072 and Dilan, and 0.5% sprays of toxaphene gave 100% immediate control of winter and black-legged ticks but only GC-4072 prevented reinfestation within 1 month.

Field tests indicated that 0.5% toxaphene sprays were slightly more effective against the lone star tick on cattle than any of the newer insecticides. However, excellent immediate control of this species was obtained with sprays of 0.025 and 0.05% diazinon and with 0.5% Dilan and V-C 13. In all cases light to moderate reinfestations occurred in 1-2 weeks. Feeding of ronnel at 7.8 mg./kg. daily for 14 days caused no reduction in tick populations on cattle.

In comparative tests 0.5% toxaphene emulsion applied with a Bean sprayer gave better control of winter ticks on cattle than when applied with a Spray-Foil machine. However, only 1 pint of emulsion was applied with the Spray-Foil as compared with about 1-1/2 gallons with the Bean sprayer.

## C. Insecticide Residue Determinations

1. Residue Studies. Work was done in Texas and Maryland on the absorption, distribution, storage and metabolic fate of insecticides in animals using chemical and radiometric methods of analysis.

In Texas a steer given 1.88 mg./kg. orally of C<sup>14</sup> phosphamidon showed peak activity in the blood in 12-18 hours, in the urine at 4 hours and in the feces at 24 hours. Little or no activity was detectible after 144 hours. Of the dose received, 71% and 5% was excreted in the urine and feces, respectively. At least 6 metabolites were found but none could be identified. Radiometric analyses did not show significant residues in muscle or fat although the method was sensitive to 1.0 part per billion.

Special tests were conducted in Texas to compare the metabolism of P<sup>32</sup> Dipterex in two cows which had shown widely variable results in tests with systemics against cattle grubs. Peak activity in the blood and urine of the two animals occurred at the same time but was twice as high in one as in the other. Over a 3-day period one animal excreted nearly twice as much activity in the urine as the other. A comparison of the metabolites indicated that one animal could destroy the less polar compounds much faster than the other. These differences in the metabolism of Dipterex indicate that the effectiveness of systemics against cattle grubs may be correlated with the animal's ability to metabolize the insecticide.

Analytical methods were developed at the Texas laboratory for determining the amounts of ronnel and V-C 13 in tissues of animals that had been treated with these insecticides. The method was based on the alkaline hydrolysis of the compounds and the determination of the corresponding phenols with amino antipyrene. Analyses of fat samples from animals sprayed with 0.5% of V-C 13 showed significant residues following spraying but after 8 weeks residues ranged from 0 to 0.05 p.p.m.

In Maryland cattle fed for 104 days on apple pomace containing 103 p.p.m. of DDT accumulated large amounts of DDT in their fat. After the cattle were placed on DDT-free feed, the levels of DDT gradually decreased but after 607 days their fat still contained 8.9 p.p.m. of DDT, which is in excess of the established tolerance of 7 p.p.m.

2. Toxicity Studies. Work was conducted in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity to livestock of insecticides and other materials applied by different routes of administration. A summary of the results are presented. Detailed results will be given under Unit 2, Animal Diseases and Parasites (ADP a7-11 and ADP a7-12). Extensive tests were run to determine the toxicity of a number of insecticides administered orally, dermally, and by intramuscular injection to cattle. In conventional spray tests with cattle, three of seven insecticides caused no symptoms of toxicity. These materials and the concentration used were Bayer 34727 at 0.25%, Stauffer R-1504 at 0.5% and Rhodia 9895 at 2.0%. One of three animals was affected by

a 0.5% application of Bayer 39193. In pour-on tests Bayer 37342 at 2.0% (125 ml.), Bayer 37342 and Bayer 34727 at 2.0% (250 ml.), and GC-4072 at 1.0% in oil (250 ml.) produced no symptoms of toxicity. GC-4072 at 1.0% in water, Bayer 37341 and Stauffer R-1504 at 2.0% affected some animals and not others. When given orally in capsules to cattle, the following materials were nontoxic: Bayer 37341 and Bayer 37289 at 10 mg./kg., Hercules 9699 at 13.4 mg., Stauffer N-3047 at 15 mg., Bayer 39193 and Stauffer N-2310 at 50 mg., and Neguvon and Rhodia 9895 at 100 mg./kg. Several other materials were toxic to some animals at the dosage tested. In similar tests with calves, 4 of 7 materials were nontoxic at low test dosages but toxic at higher dosages. No toxic symptoms were evident in cattle given 10 daily dosages of the following compounds in feed: V-C 13 at 3 mg./kg.; Neguvon at 5 mg.; GC-4072, Baytex and Stauffer R-1504 at 2.5 mg.; and Butonate and Famophos 25644 at 10 mg./kg. Butonate, Bayer 37341, Bayer 34727 and Rhodia were toxic to some animals. In additional feeding tests with horses, ENT 20738, Bayer 37341, Butonate and Ruelene were nontoxic at 50 mg./kg.

Special tests were run to determine the effects of Dibrom mist sprays as applied to the heads of cattle in controlling the face fly. Repeated applications of approximately 64 cc. of 1.25% and 24 cc. of 1.04% Dibrom produced ocular discharges in most of the animals and opaque spots on the eyes of several.

During the year a number of promising insecticides were applied at high concentrations (5-10%) with a chromatography sprayer at the rate of 100 ml. per animal. Seven compounds caused no obvious toxic symptoms but several lowered the cholinesterase (ChE) of treated animals.

# D. <u>Biological Control</u>

In Nebraska large-scale releases of pupal parasites were initiated early in June and continued until early fall at the stockyards in Omaha and in a 36 square-mile farm area near Lincoln in an effort to control stable flies. The percent parasitism was low during the first month of releases and varied greatly (0-100%) from week to week but the average level of parasitism apparently was sufficient to keep populations at a subannoying level throughout most of the season. The parasite, <u>Spalangia muscidarum</u>, was most effective in dense, compact breeding habitats whereas Muscidifurax raptor was most effective near the surface of breeding sites.

# E. Insect Sterility, Attractants, and Other New Approaches to Control

1. Mosquitoes. In Oregon studies were conducted with a number of chemosterilants. Unfed virgin female <u>Culex tarsalis</u> from 1 to 6 days old did not produce eggs after being sprayed with 5% tepa. Adults

feeding on sugar containing 0.1% apholate were completely sterilized. Females produced from larvae exposed in 1 to 3 p.p.m. of tepa laid normal numbers of viable egg masses but at 10 p.p.m. very few eggs were produced and viability was less than 1%. Adults emerging from water containing 10 p.p.m. of apholate did not lay viable eggs. Female mosquitoes were sterilized by feeding on mice which had received oral doses of 10 mg./kg. of metepa or 50 mg./kg. of apholate but lower doses were not effective. Maximum effects were apparent only in adults feeding from 15-60 minutes after the mice had been treated. Studies with a radioactive chemosterilant showed that the material was rapidly absorbed and metabolized by mosquitoes and mice. Almost complete degradation occurred in 24 hours.

Other studies in Oregon were conducted to determine the effects of irradiation on various stages of <u>Culex tarsalis</u>. Dosages required to kill 100% of the various stages were as follows: Eggs, 800-1000 r; larvae, 150,000-180,000 r; pupae 80,000 r; and adults 100,000 r. Sterilizing doses were 5,000 r for females and at least 10,000 r for males. Doses up to 15,000 r had no effect on adult longevity but 25,000 r was definitely harmful.

Approximately 200 chemicals and other materials were tested to determine whether they would repel or attract oviposition by <u>Culex pipiens</u> <u>quinquefasciatus</u> or <u>C. tarsalis</u>. Several materials attracted more oviposition than distilled water but hay infusion was the most effective. A number of materials apparently were repellent and prevented oviposition by females. The most repellent materials were emulsifiers, the best of which were effective at only 2.0 p.p.m. Over 100 chemicals and other materials, including sex extracts, were evaluated as attractants for <u>C. tarsalis</u> and <u>C. pipiens quinquefasciatus</u>. None of the materials was as attractive as the carbon dioxide standard.

2. Stable Fly. In Texas a large number of chemicals was evaluated by several methods as chemosterilants against the stable fly. The most effective sterilants were apholate, tepa, metepa and crotonamide. Topical applications of 1.8-3.7 µg of apholate to newly-emerged flies had no effect on oviposition but viability was low (0-4%). Similar applications to 6-7 day old flies reduced egg viability to 2%. The sterilizing dosage was slightly less for males than for females. Flies exposed for 48 hours on residues of 10 mg./square foot of apholate on glass oviposited normally but none of the eggs hatched. The residues were effective for 22 weeks. Flies exposed for 1-hour on a residue of 100 mg./square foot of apholate were completely sterilized. Apholate and other sterilants were more effective on glass than on wood surfaces. Stable flies fed on blood containing 0.25 and 0.5% apholate and aphoxide and 0.125% metepa oviposited normally but none of the eggs hatched. Feeding of 0.05% crotonamide did affect oviposition

but only a few of the eggs hatched and none of the larvae survived to maturity. Low concentrations of 0.001 to 0.002% of these 4 sterilants in larval media had no deleterious effect on larval survival or development. Larvae dipped in concentrations of 0.1 and 0.5% survived but their development was retarded.

In Texas selected chemicals and other materials were tested as stable fly attractants. None, including sex extracts, proved attractive.

Studies were initiated in Florida and Maryland on the development of physical and mechanical methods of controlling stable flies, with particular emphasis on radiant energy. Extensive tests were conducted to develop suitable techniques for studying the response of the light and for handling the flies. The flies were equally attracted to BLB, BL and daylight fluorescent lights. The rate of attraction was not affected by changes in light intensity. Maximum response occurred near the end of the dark part of the photoperiod, indicating that a "physiological clock" may exist in flies.

3. Face Fly. In Nebraska about 200 chemicals and other materials were tested as attractants for the face fly. None of the materials was as attractive as fresh feces of cattle. Efforts to isolate and demonstrate sex attraction were unsuccessful.

Preliminary studies in Nebraska showed that aphoxide effectively sterilized the face fly. When both sexes were fed sugar containing 0.25% aphoxide for one day or 0.0025% for three days oviposition was reduced and the eggs did not hatch. Normal females mated with males fed for 3 days on 0.005% aphoxide produced only non-viable eggs.

4. Screw-worm. In Texas, where investigations on the screw-worm were conducted, over 250 compounds were screened as chemosterilants by several methods against various stages of the insect. About 50 of the materials caused complete sterility by one or more methods of test. Nearly all of the effective sterilants were confidential materials which cannot be identified by name or structure at this time. Known materials causing complete sterility were: Apholate, tepa, tretamine and metepa, applied topically and in adult food; colchicine, 2,6-diaminopurine and morzid, in food; and methiotepa and Thiotepa, applied topically. One material (confidential) was effective as a sterilant in the larval media but was ineffective by other means of application. Some of the active materials sterilized either sex, others were effective only when both sexes were treated, and others were effective only on one sex. Tretamine and a number of other materials sterilized all ages of flies but some materials were effective only against newly-emerged flies.

Tests indicated that there was no consistent difference in the effects of 1,000 and 5,000 r administered as a single dose or two equal doses. Female flies 3, 4, and 5 days old differed greatly in radiation sensitivity but dominant lethals were induced in the oocytes of all ages. In additional tests with 5-day-old pupae, which have only oogonial cells, the number of eggs deposited by females decreased as the radiation dose increased, indicating that damage to some cells eliminated them from the germ line and that they were not rapidly replaced.

Over 200 chemicals and other materials were screened for attractiveness to the screw-worm fly by special olfactometer procedures and by exposing them in beakers in cages of flies. In olfactometer tests isovaleral-dehyde was 10 times as attractive as liver (standard) but was less attractive than liver in beaker tests. Several additional materials were as attractive as liver but none was superior. Studies with liver and other materials indicated that light increased attractiveness and that maximum attraction occurred at 95°-106° F. Materials exposed at 86°-113° attracted 2-3 times as many flies as when exposed at 67°-80°. Very few flies from 1 to 3 days responded to attractants. Highest attraction occurred when flies were 3-4 days old.

5. House Fly. Studies in Oregon indicated that irradiation of resistant house flies with sub-sterilizing doses of 1,000 r did not alter their susceptibility to insecticides or esterase activity. Treated females mated with untreated males oviposited normally but only 45% of the eggs hatched. Additional studies were conducted with normal and parathion-resistant flies that had been irradiated as puape with 600 r for seven generations. Only about 25% of the eggs hatched from matings of irradiated males and females of either colony. No changes in insecticide susceptibility or esterase activity were apparent.

Extensive research on sterilization, attractants and other new approaches to control of the house fly was conducted at Orlando, Fla., in connection with the control of the house fly in dairy barns, poultry houses, households and industrial establishments. Since the results of this research is applicable to the control of house flies in other situations, some of the highlights of recent research at that laboratory follows.

Irradiation caused greater damage to early (2-4 days old) house fly pupae than to middle-age pupae. Virtually no damage was apparent in old pupae and longevity of adults was greater than that of younger pupae. Irradiation of pupae 0-36 hours before adult emergence did not produce complete sterility and some recovery was indicated since second matings resulted in a slight increase in progeny. No recovery occurred in pupae irradiated 72-96 hours before adult emergence.

Extensive studies were conducted to find materials that will induce sterility or otherwise affect the growth and development of the house

fly. Of about 1300 compounds tested in the adult food, 21 caused sterility in flies. Only one of about 800 materials tested in the larval media caused sterility but nearly one-third were toxic to the larvae. In secondary tests with 59 compounds that had shown promise in screening tests, twenty-seven caused sterility (no oviposition or hatch) at concentrations of 1% or lower. Additional tests were run with 50 promising materials applied as larval dips, topically to adults, and in the adult food. None caused sterility as larval dips but in adult food, six induced complete or nearly complete sterility. Two other materials were effective only as topical applications.

Special tests were run with 24 promising chemosterilants to ascertain the effects of each sex. Only one material, an aziridinyl compound, caused sterility in both sexes. Methiotepa, 5-fluoroacetic acid, metepa and a confidential material caused complete sterility in males but not always in females. Three materials, 5-fluoroacetic acid, an aziridinyl compound, and 5-fluorouracil, were effective when fed to both sexes.

Studies were conducted to learn more about the action of several effective chemosterilants on house fly sexual development, mating and reproduction. In one series of tests, males sterilized by feeding 3 days on apholate proved fully competitive with normal males when placed with normal females. When only treated males were placed with normal females all eggs were sterile and 12.5% were sterile when only normal males were present. When normal females, normal males, and treated males were combined at ratios of 1:1:1 and 1:1:2, 65 and 80% of the eggs were sterile and higher ratios of 1:1:3, 1:1:5, and 1:1:10 resulted in 99.9 to 100% sterility. Additional tests at these ratios confirmed that actual sterility was higher than expected. Males given food containing 0.4 to 1.0% apholate for 3 days were sterilized for life but lower concentrations of 0.1 to 0.3% were not 100% effective. Exposures of males on residues of 500-1,000 mg./kg. of tepa on plywood panels caused only partial sterility (12-72%) but when applied at 250-500 mg./square foot with sugar tepa and apholate produced 91-100 and 99-100% sterility, respectively. When applied in glass jars, residues of 250 mg./square foot of tepa or metepa completely sterilized flies in 2-4 hour exposures for 30 days but not for 60. Deposits of 100 and 50 mg./square foot caused complete sterility for 14-30 and 14 days, respectively, but deposits of 10-25 mg. were mostly ineffective. containing 0.5% of tepa and metepa effectively sterilized flies after aging 30-37 days on most types of surfaces. Some loss in effectiveness in tepa was apparent on metal and masonite and in metepa on wood but none was apparent on painted wood, asphalt, metal or wax paper.

Weekly applications of cornmeal bait containing 0.5% tepa on an isolated refuse dump reduced adult house fly populations from 47 to 0 per grid count in 4 weeks and counts remained at 0 as long as the

bait was distributed. The viability of eggs of female flies declined from 100 to 10% in 4 weeks and to 1% in 5 weeks. After baiting ceased, populations increased slowly but the percent viability of eggs was normal after 2 weeks. Additional small-scale field tests with cornmeal-chemosterilant baits against flies were conducted on a small garbage dump and in a poultry house. Weekly applications of apholate baits on the dump and of metepa baits in the poultry houses caused some sterility and reduction of fly populations. Applications 5 days a week resulted in a high degree of control and high sterility in flies in both areas. Sterility among females was slightly higher than in males.

Comparative tests showed slight differences in the competitive ability of male flies given 1% apholate in food and those irradiated with 2,850 r. Neither radiation nor the apholate completely sterilized the males but at a 4:1:1 ratio chemosterilized males caused a reduction in egg hatch of 81.4% as compared with 78% for the irradiated males.

6. Ticks. Preliminary tests were conducted in Texas to study the effects of several known chemosterilants on tick molting, longevity, and reproduction. Engorged lone star tick larvae dipped in 1.0% apholate molted to nymphs and then to adults but those dipped in 0.5% tepa, tretamine and metepa failed to molt to nymphs. In similar tests in which engorged nymphs were dipped in 0.5% solutions the percentages molting were as follows: Apholate, 45%; tepa, 70%; and tretamine and metepa, zero. All unfed female ticks dipped in 1.0% solutions of these four materials failed to engorge. From 40-60% of those dipped in 0.5% solutions engorged but data are not yet available on the sterilizing effects of the materials. The females dipped in apholate required 15.5 days to engorge but those dipped in other materials engorged in about the same time (11.2 days) as control females.

Studies in Texas indicated that irradiation at the rate of 1,000 r had no effect on the molting of unfed or engorged nymphal lone star ticks. However, a dose of 2,500 r prevented molting of unfed and 1-day engorged ticks and only 3% of those engorged 1 week molted to adults.

## F. Evaluation of Equipment for Insect Detection and Control

1. <u>Sprayers</u>. In Texas, in cooperation with the Agricultural Engineering Research Division, one series of tests was conducted to compare the efficiency of a conventional (Bean) sprayer with that of a Spray-Foil sprayer in applying insecticides to cattle. The Spray-Foil machine gave slightly less control of ticks than the conventional sprayer. However, the Spray-Foil machine applied only one-tenth as much spray per cow as the conventional sprayer. With an increase of two-fold in output the Spray-Foil machine would probably give as good results as the conventional sprayer.

2. Mechanical Devices. In Maryland studies were initiated to evaluate available models of light traps, insect electrocutors and other mechanical devices for the control of flies and other insects. Primary emphasis has been given to comparing the attractiveness of different kinds of light and different intensities. Special test chambers were developed for this purpose. None of the devices tested proved highly attractive or effective. Further tests of existing equipment and efforts to develop more efficient traps and other devices are in progress in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions of ARS.

## G. Insect Vectors of Diseases

Anaplasmosis. Studies were continued in Mississippi, Texas and Oregon in an effort to correlate the presence and abundance of insects and ticks with the incidence of anaplasmosis in herds of cattle. studies were conducted in cooperation with the Animal Disease and Parasite Research Division and veterinarians of the various State Experiment Stations. In Mississippi, daily applications of synergized pyrethrins sprays (0.05% pyrethrins / 0.5% synergist) with an automatic sprayer gave complete control of horn flies and significantly reduced attacks by horse flies. The effectiveness of the spray was reflected in a very low incidence (4 cases) of anaplasmosis in the treated herd as compared with that (18 cases) in the control herd. The 4 cases in the treated herd were mild, whereas there were a number of acute cases in the control herd and two animals died. The fact that first transmission in the treated herd did not occur for two months after the first case developed in the control herd serves as a further indication of the effectiveness of the sprays in protecting animals from biting In another test, daily feeding of aureomycin at the rate of 0.5 mg. per pound of body weight reduced the transmission of anaplasmosis even though no effort was made to control biting flies. Only 4 cases of the disease developed in the antibotic herd compared with Il in the control herd. The first case in the treated herd did not occur for over two months after the first one in the control herd.

In Texas monthly surveys were continued to determine the identity and abundance of external parasites on infected (anaplasmosis) and isolated clean herds of cattle. Small numbers of lone star ticks, ear and black-legged ticks and moderate numbers of the winter tick were present on cattle in January. Populations of the lone star tick increased steadily during February and March, but populations of other ticks were low. First horn flies appeared in March. During April, May, June and July, cattle were heavily infested with the lone star ticks and horn flies and with small to moderate numbers of ear ticks. Populations of all these species were low during August and September. In October the winter, ear and black-legged ticks and horn flies and grubs were

present in small numbers. Moderate to high populations of the winter and ear ticks and grubs and small numbers of black-legged ticks were noted during November and December but no flies were observed on the cattle. No transmission of anaplasmosis has occurred in the isolated clean herds although no effort has been made to control potential insect and tick vectors.

The Oregon station continued surveys in Wyoming from spring to fall to determine the distribution, abundance and seasonal occurrence of potential arthropod vectors of anaplasmosis on several experimental herds of cattle on the Myers' Ranch. The tick, <u>Dermacentor andersoni</u>, a known vector of anaplasmosis, appeared early in the spring, reached a peak population in May, declined gradually thereafter, and virtually disappeared by August. Light to moderate populations of several species of lice were present on cattle throughout the season. Horn flies and numerous species of mosquitoes were present in small to large numbers from May until the advent of cool weather. About 12 species of horse flies and deer flies were present in small numbers throughout the summer. Negative susceptible cattle have developed very few cases of anaplasmosis despite the presence of ticks and other vectors. It therefore appears that natural transmission of anaplasmosis rarely occurs under the Myers' Ranch conditions.

Studies were continued at Beltsville, Md., on the transmission of bovine anaplasmosis. Further attempts to demonstrate transovarian passage of the anaplasma agent in <u>Dermacentor andersoni</u> were negative When unmated males were forced into hibernation, the survivors readily transmitted the disease 6 months and 3 weeks after engorging on infected cattle. The unmated males survived longer than mated males under hibernation and normal colony conditions. None of the mated males survived hibernating conditions. A series of <u>D</u>. andersoni specimens taken from cattle in the experimental areas on the Myers' Ranch in Wyoming were tested on splenectonized calves. None of the ticks transmitted anaplasmosis.

Progress was made in studies on the anaplasmosis organism in ticks, using fluorescent antibody, electron microscopy, and conventional staining and histological techniques. Structures believed to be the projection part of the organism were demonstrated in the gut and feces smears by the fluorescent antibody technique. These structures were also found by electron microscopy in feces smears.

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EQUIPMENT AND BUILDINGS USED IN PRODUCING BEEF CATTLE AND HOGS Agricultural Engineering Research Division, ARS

Problem. Economic conditions are causing farmers to step-up their efforts to reduce production costs and improve quality by reducing labor and modifying environment in livestock production.

Labor is an important element in production costs. How to make better use of equipment and to adapt existing buildings and other facilities for more efficient production as herds are increased in size and farms consolidated are major considerations. Cost of replacement or major improvement of existing buildings that are not suited to modern production methods are serious obstacles. Principles, examples, and techniques for planning more efficient operations are needed both by farmers doing their own engineering and by those on whom farmers depend for advice.

Today's technology in farming, as well as research, requires accurate instruments for measuring or monitoring processes such as grain and forage drying and plant and animal environment. Current agricultural research is especially dependent upon accurate instrumentation; some problems require completely new kinds of instruments. Studies are necessary to determine the accuracy and practicability of instruments for many kinds of agricultural measurements.

### USDA PROGRAM

This is a continuing program involving engineers and architects conducting basic laboratory investigations, application of laboratory results to a production basis, and development of typical plans for livestock structures. The work is in cooperation with the AH, ADP and ENT Divisions of ARS, USDA, and State Agricultural Experiment Stations, and contributes to Cooperative Regional Projects NC-23, "Farm Structures to Meet Environmental Requirements of Dairy Cattle, Swine, and Poultry," S-49, "Genetic Methods of Improving Dairy Cattle for the South," and NE-8, "Essentials of Poultry Housing for the Northeast." Plan development work is cooperative with all the State Agricultural Experiment Stations and Extension Services.

Beef cattle structures and equipment research for hot, dry climates is conducted in cooperation with the California Agricultural Experiment Station at the Imperial Valley Field Station, El Centro. Related studies in a warm, humid climate are in cooperation with the Georgia Agricultural Experiment Station at Tifton, with AH, ARS, advice. Typical plans for beef structures are developed at Beltsville. This work involves 1.6 professional man-years.

Swine structures and equipment research is in cooperation with the California Agricultural Experiment Station. Methods of modifying the thermal environment are being investigated in field studies at Davis and at the Imperial Valley Station, El Centro. With the additional cooperation of a hog producer, the influence of building type and arrangement on efficiency of animal growth is studied on a production scale. Studies of the effects of floor space per animal on growth and feed consumption have been concluded. Typical plans for swine structures are developed at Beltsville. This work involves 1.0 professional man-years.

Equipment and control for automatic feeding of livestock and poultry is under development in Washington and Illinois State Experiment Stations. Work on performance characteristics of upright-silo unloaders is in cooperation with the Minnesota State Experiment Station. The Federal scientific effort devoted to research in this area totals 6.6 professional man-years, of which 3.6 is devoted to equipment for livestock.

Work on electric and solar equipment for environmental control involves 0.2 professional man-years for swine environment equipment.

Reduction of pesticide residues in animal products, with beef cattle receiving major attention, is studied at Kerrville, Texas, in cooperation with ENT and ADP, ARS, and the Texas Agricultural Experiment Station, involving about 1.0 professional man-year.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

The experiment stations in 1961 reported the following professional manyears of effort: beef buildings, 2.2; swine buildings, 4.5; beef equipment, 1.3; swine equipment, 0.4; and an estimated 4.3 professional manyears of research on equipment for environmental control in livestock buildings; beef equipment, 1.3:

Beef building research is in progress to determine the relation of animal productivity to modified local climatic environment. Measurements of heat and moisture loss from beef cattle under various stress conditions are being determined and these data are being used in the design and construction of structures to provide optimum environmental conditions.

Swine building studies to determine the relation of climatic factors to the physical well-being of swine are in progress. These data are being directly applied to problems of design of structures and equipment for optimum economic production. Special investigations are underway to evaluate effects of combinations of temperature and humidity on growth, reproduction and efficiency of production. These results will assist the establishment of design criteria for environmental controls and equipment for swine housing units. These studies also are a part of Cooperative Regional Project NC-23.

Part of this research is cooperative with the USDA. Investigations to analyze and to develop and test equipment for systems to mechanically feed and service beef and dairy cattle, swine, and poultry are in progress. These studies involve the design and installation of automatic feeding systems for blending mixed rations and roughages according to various levels of production and the removal of waste materials. cluded also are investigations to determine the necessary specifications and functional requirements for improvements in a mechanical system for removal of chopped hay and silage from storage and its transport for animal feeding. These studies are a part of cooperative regional projects NE-13, "Studies of the physical properties, behavior, and forms of forage as related to engineering application," and NC-48, "Development of materials handling systems for North Central farms." Research is underway to determine the operating characteristics of various conveying systems and forage and silage unloading devices and to determine the functional requirements and design of automatic controls for an integrated system for livestock feeding operations. The primary objective of these studies is to arrive at suitable methods and equipment to reduce the labor now required and thus reduce unit cost of production. Some similar work on equipment for swine, poultry, sheep, bees, and milk equipment is conducted in various states.

In industry several manufacturers of metal and other types of prefabricated buildings have been actively testing the performance of their buildings for housing dairy and beef cattle, swine and poultry. Estimated annual expenditures in 1961 were equivalent to approximately 20 professional man-years. Manufacturers of lumber, cement and other materials have been developing new materials, investigating their applications and determining construction techniques for their use. However, most of this research has been directed to supplying the larger markets provided in industrial structures and non-farm dwellings. Estimated annual expenditures directly benefiting farms in 1961 were equivalent to approximately 30 professional man-years.

Most manufacturers of "on-the-farm" equipment for livestock and poultry are engaged in testing the performance of their product design and developing improved products. Some are also investigating farm application of products designed for other uses and a lesser number are developing new equipment or methods to meet specific problems in the livestock and poultry industry on farms. Feed grinders suitable for hard corn in Kansas, for example, are not necessarily satisfactory for soft corn in Minnesota. Research is often conducted on a cooperative basis with electric utilities and with State experiment stations to save costs and to obtain nationwide results in testing equipment under a variety of conditions and crops. Industry maintains close contact with USDA research for information on functional requirements and performance characteristics for electric motors, equipment and controls;

for example, the motor and control requirements for silo unloaders. The estimated annual industry expenditures for research on items specifically for "on-farm" use are believed to be approximately 10 manyears on poultry equipment, 10 on beef equipment, 10-15 on dairy equipment, 5-10 on swine and less than 5 on sheep equipment.

A number of companies are engaged in the design, development and adaptation of instruments for agricultural applications. These are mostly instruments that can be used in warehouses, processing plants, and laboratories for routine testing and measuring. Most instruments for basic farm research and farm use are of a specialized nature and quantities required are not sufficient to induce industry to conduct research or develop them. The estimated annual expenditure of research on instrumentation specifically for farm research and farm use is believed to be equivalent to approximately 5 professional man-years annually.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

## Corral, Shade and Cooling Studies with Beef Cattle.

A study begun in 1959 in cooperation with the Imperial Valley Field Station, El Centro, California, used 12 pens and 5 animals per pen. The animals used in the 1959 and 1960 tests were Herefords obtained in the Imperial Valley; the 1961 animals were Herefords from the University's range 40 miles north of Davis. The treatments were: 90, 135, and 200 square feet of pen area per animal; 27 and 48 square feet of shade per animal; and, high- and low-energy rations. Previous studies have shown the need for shade in the Imperial Valley. The feeding trial was 84 days when the mean temperature was 90.7° F. and mean air velocity 2.2 mph. Initial weight per animal was about 540 pounds.

Combined analysis of the data for three years indicated for average daily gain:

- (a) No difference between 27 and 48 square feet of shade.
- (b) No difference between 90, 135 or 200 square feet of pen area.
- (c) Highly significant greater gains on high-energy rations.
- (d) Highly significant lower average daily gains in 1961 compared with 1959 or 1960.
- (e) No significant interactions between any of the factors studied.

It is therefore concluded that (a) during most years, an average shade area of 25 to 30 square feet per animal would be adequate for very hot dry areas such as the Imperial Valley; (b) total pen area as low as 90 square feet per animal is not so restrictive as to limit feed intake or average daily gain.

Basic investigations at El Centro on the effect of exterior surface cooling of beef cattle on weight gains and physiological responses yielded no conclusive findings and will be continued. A "head cooler" feed bunk 12 feet long, enclosed in a box to permit recirculation of cooled air, was constructed and fitted with 5 stanchions so the animals could be locked in when necessary. Five Hereford steers ate from this during the 84-day beef cattle test period. Although the equipment did not function as planned and the design air temperatures were not maintained, gains averaged 2.24 pounds per day, and were comparable to the gains of all the other beef cattle on test during the period.

Field evaluations of shades for beef cattle on pasture and in dry lot at Tifton, Georgia, were made in 1960 in cooperation with the State Station. Weight gains of 36 steers, weighing about 644 pounds initially, were observed during the 154-day fattening period, April 19 to September 20. Four lots of cattle were used, two in dry lot and two on pasture. There was a shade in one dry lot and one pasture. Steers in all lots received full feed of ground snapped corn and cottonseed meal; those in dry lot received Coastal Bermuda hay. The pasture was Coastal Bermuda grass. Cattle in the pasture with shade made slightly higher gains than those without shade, but this finding was reversed in the dry lots.

Growth rates of 24 steers grazing on pasture, with and without shade, were observed during the same 154-day period. The study did not show that the shades increased the rate of gain under Georgia conditions which are much less severe than those at El Centro.

Typical plans for corral and feed lot, dipping vat, and pen for truck scales specifically for beef cattle were developed at Beltsville for the Cooperative Farm Building Plan Exchange. Other plans for dairy or beef cattle include feeding and watering equipment, and hay storages and feeding shed.

Pen Space Requirements for Confinement-Raised Hogs. In the University hog barn at Davis, space allotments of 5, 10, and 20 square feet per pig (excluding self-feeder space), and group sizes of 3, 6, and 12 animals per pen, were used in a 3 x 3 factorial design. There were 3 pigs per pen in pens of 15, 30 and 60 square feet area; 6 pigs per pen in pens of 30, 60 and 120 square feet area; and 12 pigs per pen in pens of 60, 120 and 240 square feet, respectively. Automatic waterers were used, one feeder opening for each three pigs was allowed, and initial weight of the pigs was about 80 pounds. Pigs with 20 square feet each gained weight more rapidly than those confined to 5 or 10 square feet (P < .01) Pigs with 3 per pen consumed more feed daily than either 6 (P < .05) or 12 per pen (P < .01) Pigs with 5 square feet each required more feed per unit gain than those with 20 square feet (P < .01)

with either  $5 (P - .01)^{1/2}$  or 12 per pen  $(P < .01)^{1/2}$ . Curves have been plotted from these data showing optimum space, depending upon feed and building costs.

Temperature Requirements and Control for Hogs. Studies to determine the benefits of sprays and wallows on weight gain of swine were conducted at Davis, California, in the summers of 1960 and 1961. The 1961 study was conducted in five concrete-floored pens from June 21 to August 27. Sprays were operated from 10 a.m. to 8 p.m. The respective weight gains (lb./day) and feed conversion (lb. feed/lb.gain) were: Control, 1.29 and 3.75; unshaded wallow, 1.39 and 3.68; shaded wallow, 1.56 and 4.16; unshaded sprays, 1.53 and 4.24; shaded sprays, 1.52 and 3.86. Difference in gain at 1 percent level was significant only between controls and all other treatments. At 5 percent, the gains of the control and unshaded wallow pens were significantly less than all other treatments. Average temperature and relative humidity during the period were 77.1° F. and 49.5 percent. The maximum and minimum temperatures were 96.8 and 60.1° F. and the corresponding relative humidities 26.0 and 76.6 percent.

Similar results with wallows were obtained in 1960. Cooling the wallow water about 17° F. by passing it through a cooling tower provided no significant benefit over uncooled water.

Daily observations of behavior of growing pigs subjected to 4 heat relief measures at Davis, California, yielded the following information. Observations were made at 2-hour intervals for a 50-day period.

Where shade was the only relief measure it was used about 80 percent of the time from 7 a.m. to 3 p.m. Addition of a wallow reduced the time spent in the shade. Use of portable houses (3-sided, wooden floor, metal roof) was relatively low as compared to fabric shades and other shade on the concrete floor. Use of heat relief measures increased rapidly when the temperature went above 70° F. The air-conditioned house was used about 60 percent of the time during hours of observation. An increased weight gain in all treated lots was not correlated with any behavior pattern.

The importance of suitable shade on summer days with temperature over 70° F. is indicated. The use of other relief measures such as wallows also is of importance.

An effort to duplicate in the field the weight gains of swine raised at optimum temperature growing conditions within the laboratory, was made at El Centro, California, in 1960 and 1961. In 1960, pigs in each of two open pens had access to 6' x 10' x 4' high insulated buildings 1/ One chance in a hundred of not occurring this way.

2/ Five chances in a hundred of not occurring this way.

that provided a 75° F. environment. Temperature in one house was maintained by blowing refrigerated air through it (no recirculation). Water-cooled ceiling and wall panels cooled the other. Air velocity through the air-cooled house was about 100 fpm; in the radiant-cooled house it was essentially nil. Rate of growth and feed consumption of pigs (8 per pen) with free access to these houses were compared to those of pigs with access to a shaded wallow during an 84-day summer test. Average weight of the pigs was about 80 pounds at the start of the test on June 27. Rations were self-fed outside cooling structures.

Average maximum, minimum, and mean temperatures during the period were 107.7, 75.6, and 90.7° F. Average daily gains were 1.42 pounds (air-cooled house), 1.36 (panel-cooled house), and 1.49 (shaded wallow). The respective feed consumption per unit of gain was 4.12, 3.97, and 4.00 pounds. The differences between means for average daily gain, carcass yield, backfat thickness, or average daily feed consumption were not significant. It has been suggested that the change in environment was too great when the animals moved out to eat. An activity check indicated that the house animals ate mostly between 4 and 8 a.m., whereas wallow pigs seldom ate between 7 p.m. and 7 a.m.

In 1961, the houses were lengthened from 10' to 14' to make room for a feeder and water cup in one of them. The pigs in the other house had to leave the cooled house for feed and water. Each house was used by 8 pigs from June 22 to September 6. A shaded wallow was used in the control pen. Average period temperatures were: Outside, 89.3; cooled house with feeder inside, 72.3; cooled house with feeder outside, 69.7. Differences in daily gains, yield, and backfat were not statistically different. The actual gain of the pigs in the cooled houses averaged 1.62 lb./day and that of the control pen averaged 1.52.

A series of feeding trials, in cooperation with a producer near Escalon, California, was started in a field study of three different types of housing and three or four levels of feeding. The housing is a McMaster house with insulation and evaporative cooling, a McMaster house with no sidewalls but an insulated roof and an open shed with aluminum roof and open side south. In each house several levels of feeding were used -- 90, 81, 73, or 65 percent of the ration suggested by the National Research Council. After the first test the 65 percent level was discontinued. For each test, each pen had 10 pigs that were started at about 100 pounds and fed to market weight. It is planned that these tests will cover each season of the year. The first test was started March 23, 1961, the second on July 14, and the third on December 27. The latter test ended March 26, 1962, and the results are not included in this report. For the first two tests there was no significant difference due to housing, but results of all levels of feeding were different from all other at the 1 percent level, with the greatest gain due to

the highest level of feeding, but a few pounds less feed required per 100 pounds of gain at 73 percent of the National Research Council ration level. These tests will continue.

Cattle Feeding Equipment. In Illinois the control system of the automatic beef feeding system has been modified this year by provision for adding high moisture shelled corn to the ration. Silage, high moisture shelled corn, and a ground feed concentrate can be blended to form either of two rations for automatic feeding, each to one of two lots. The winch control relays for the silo unloader have been modified to give fast-make, slow-break, contact action. This prevents the unloader from "hunting." The rate of discharge of silage from the silo by the automatically controlled silo unloader is controllable from 60 to 170 pounds per minute with a variation of 5-10 pounds/minute in 300 lb. samples. A variable orifice, constant speed auger meter tested in the high-moisture shelled corn storage tank was unsatisfactory. The soft shelled corn was crushed by the auger; the crushed corn impeded the flow of grain through the metering orifice and resulted in an erratic flow of material from the storage bin.

In Washington a low power electrically driven silage cutter unit for use in unloading trench silos has been developed and successfully tested in both chopped and unchopped grass and pea vine silage. The cutter uses two 12 or 16 inch counter-rotating augers 25 inches long. The cutter operates perpendicular to the silage face traversing from left to right and top to bottom in successive cuts. The cutter unit requires two horsepower to remove four to six hundred pounds of silage per minute. The cutter is the first step in the development of an automatic trench silo unloader. A public patent will be sought to protect this development.

In Minnesota the silo on the premises of the Agricultural Engineering Farm was used for comparing several motors on one make of unloader. These tests pointed up the difficulty of adequately protecting against burn-outs in motors designed for high torque and short duty cycle. The use of ammeters along with built-in motor protectors and current-sensitive relays appears to be most suitable. The tests were a factor considered by one manufacturer in deciding to redesign his motor for this application.

One reason farmers purchase silo unloaders is to save time and labor. This objective has not been entirely attained. Lowering of some types is still done manually which requires the presence of the operator. Two silos were equipped with small gearhead motors belted to the winch of each unloader. Mechanical lowering resulted in a very uniform load on the unloader. Adjustment of the pulley drive ratio had to be made to compensate for the decreasing diameter of the winch drum and cable. Other controls are necessary to prevent stalling.

Hog Equipment. Cooperative work is currently being carried out with the Agricultural Engineering and Animal Science Departments of the Illinois Experiment Station on automatic feeding of hogs at definite time intervals and varying amounts of feed in comparison with self-feeding. Two new experimental dump tube feeders, electrically driven and time controlled, are available for these studies.

Swine Environmental Equipment. In cooperation with the Virginia Agricultural Experiment Station a study was initiated utilizing heat pumps for determining the optimum equipment requirements for efficient methods of producing hogs from weaning to market weight. The facilities include a 24 x 60 ft. temperature-controlled building and a half-open building that have identical concrete floors and pen areas; and an open feed lot for a check. Studies on the space per animal are being made in the pens on the concrete floors. Carcass quality analyses, as related to the production facilities, are also being determined.

This project involves one summertime and one wintertime testing period per year. Each period requires 7 groups of 15 animals, 3 in each of the buildings and 1 in the open feed lot. The animals subjected to the first summertime testing used primarily as a checkout period for equipment and instrumentation were slaughtered and subjected to carcass analyses at the Virginia Agricultural Experiment Station Animal Processing Laboratory. Data are being statistically analyzed. A wintertime testing period is currently in process.

Several tests will be required to confirm results that may be notable.

An automatic feeding system has been designed and will be installed prior to the 1962 summertime testing period. A lagoon waste disposal system for the facility is in use.

Basic studies on the effects of variously charged air particles on hogs were continued at Davis, California, in cooperation with the State Station. Durocs, in groups of four, were subjected to one- or two-week periods of either normal, excess positive or excess negative ions. At 90° F. weight gains and feed conversions have been quite inconsistent except that, in all cases, one of the ion treatments has resulted in improved gain and conversion. More data are required and "ion houses" have been constructed for this so that pigs can be subjected to the three conditions simultaneously. Ozone testing equipment was designed and built and is now being used to determine ozone concentrations.

Efforts to develop cooling indices from records of power used by air-conditioning units, produced quite good correlations. Several years' records were collected at Davis, California, on the amount of power used by air-conditioning units on turkey pens (housing caged birds) and

swine houses used in other research. These records were analyzed at Columbia, Missouri. The indices used so far include cooling degree-days based on the daily mean temperature above 60° F. and on the Temperature Humidity Index above the value of 60. Correlations of these indices were quite good for the data analyzed to date. It is possible that use of computer facilities will develop an even better cooling index, and also allow pooling of data from the various units to provide prediction of operating costs beyond the limitations of the actual air-conditioning units for which data were recorded.

Research Instrumentation for Livestock. Ultrasonic pulse-reflection equipment provides a method for measuring, without penetration, the thickness of farm animal fat and lean tissues. Studies to improve method accuracy, and to apply the ultrasonic measurements to animal composition research were continued in cooperation with the Meat Quality Laboratory, Animal Husbandry Research Division, at Beltsville. The presence of an additional (third) fat layer at the longissimus dorsi ("loin-eye") measuring point off the midline was shown on market weight hogs. The measuring method was changed to include this layer. Measurements on pieces of hog fat of known thickness showed no significant differences due to variations in sound velocity.

New measuring points were added to provide further reliable estimates of animal meatiness. These points in hogs are at the juncture of the ham and loin, and over the 13th rib, 10, 12 and 14 inches off the midline. New points in cattle were at the juncture of the 13th rib and its cartilage attaching to the sternum and over the short loin, one-third the distance from the 13th rib to the pinbone.

A preliminary statistical analysis indicated that a combination of live weight and ultrasonic fat and lean measurements could be quite successful in predicting yield of hind quarter high-priced cuts of beef. (Correlation above 0.9.)

Animal holding methods and check measurement reliability need improvement before other significant factors can be studied to further increase the accuracy of the ultrasonic method. Ultrasonic pulse-reflection has shown utility as a basic research tool. However, the method is still in the development state.

Studies were conducted of relative humidity recorders (hair type) to determine probable error under poultry house constant temperature with humidity varying. Instruments were calibrated prior to and following studies in precision chamber. Instruments in new condition recorded humidity with errors less than \$\frac{7}{20}\$ percent relative humidity at the calibrated temperature. At varying temperatures the error was doubled (\$\frac{7}{20}\$ percent relative humidity).

Bio-electronics in Animal Slaughter. In cooperation with the Meat Quality Laboratory, apparatus was assembled by AERD and techniques developed for monitoring of physiological reactions of animals during humane slaughter procedures. Based upon fundamental studies and information derived from contract studies at the University of Minnesota, procedures are being established to determine practical parameters of mechanical stunning during pilot slaughter operations.

Reducing Insecticide Residues in Animal Products. At Kerrville, Texas, equipment and procedures for fundamental studies on reducing chemical hazards associated with the control of livestock insects are being developed, in cooperation with ADP and ENT, ARS.

A metal building, 36' x 60', to house shop, office and laboratory was completed and equipped in January 1962.

Research studies were then started to determine the relation between the deposition of insecticides on animal hair coats and spraying and dusting variables such as particle size, particle velocity, spray viscosity, surface tension and others. A preliminary test indicated that only about 25 percent of a one-gallon application of water containing 2.3 percent copper sulfate will remain on a Hereford cow even when the cow has a winter hair coat. As this work progresses, effects of animal characteristics such as length, weight and density of hair coat will also be considered.

Other studies have been set up to determine methods of measuring and controlling the distribution and uniformity of insecticidal depositions on livestock.

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# II. UTILIZATION RESEARCH AND DEVELOPMENT

MEAT - PROCESSING AND PRODUCTS
Eastern Utilization Research and Development Division, ARS

Problem. Livestock production is the greatest single source of farm income: production of meat animals accounted for 31 percent of farm cash receipts in 1960, and the major part of U. S. farm land suitable for cultivation is used to produce feed for livestock. Hence any research which succeeds in stimulating an increase in the consumption of meat and other livestock products can provide a powerful stimulus to U. S. agriculture. For example, a one percent increase in meat consumption would require feed equivalent to about 80 million bushels of corn.

Increases in livestock consumption may be achieved through development of new or improved meat products, or through improved meat processing technology which results in lower costs. In addition, increases in the value of hides, animal fats and renderers' proteins will benefit the livestock industry by providing additional revenues which could permit reduction in meat prices (thus stimulating consumption) or which could flow back through the marketing channels in whole or in part to livestock growers and feed producers. For example, it is estimated that loss of the market for hides would cause an increase of meat prices that would result in a decrease of 2 percent in meat consumption. Such a decrease would eliminate a market for feed equivalent to 160 million bushels of corn. Conversely, an increase in hide values would operate in the opposite direction and would result in greater income to the livestock industry and in increased utilization of feed grains.

The attainment of an increase in livestock consumption requires both applied and basic research. Applied research is the forerunner of commercial practice and is an indispensible element in successful development. But applied research is based on the fundamental knowledge that is acquired through basic research, and represents the exploitation of this fundamental knowledge. The supply of fundamental knowledge must be maintained and expanded, if applied research is to be most effective and fruitful. The need for basic research has been pointed out by the Commission on Increased Industrial Use of Agricultural Products, by the National Agricultural Research Advisory Committee and by other responsible groups.

For the reasons outlined above, research which succeeds in increasing meat consumption can have a powerful effect on American agriculture. The potential effect may be assessed from the facts that (1) meat has a high elasticity of demand (a 1 percent drop in retail meat prices will result in a 0.7 percent increase in

consumption); (2) the production of 1 pound of livestock requires the equivalent of 7-8 pounds of feed grains; and (3) the U. S. per capita consumption (160 pounds) is no higher than it was 50 years ago and is below that of several other countries, including Australia (234 pounds per capita), New Zealand (222 pounds), Uruguay (234 pounds) and Argentina (166 pounds).

Increases in meat consumption may be achieved by the development of new and improved products that will stimulate demand, or by improvements in processing technology that will lead to reduction in meat prices. The effect of lower prices on meat consumption and on feed utilization is especially striking. Thus, a 1-cent-per-pound reduction in retail meat prices should lead to an increase in consumption of about 1.1 percent. The additional production required to meet this increase will require feed equivalent to about 85 million bushels of corn-the output of about 1.6 million acres.

The attainment of increased consumption will require a vigorous and balanced program of applied and basic research. There is a need for more applied research on processing and preservation, including time-temperature studies of frozen meat, and on new and improved meat products. Of equal or greater importance is the need for more basic research on the physical, chemical and organoleptic characteristics of meat, and the microbiology of meat.

#### USDA PROGRAM

The <u>Department</u> has a continuing program involving chemists, biochemists, and microbiologists engaged in both basic studies and the application of known principles to the solution of problems in the processing of meat and its products. The Department's research facilities are located at Beltsville, Maryland.

The Federal scientific effort devoted to research in this area totals 15.8 professional man years. This effort is applied as follows:

- (a) Chemical and physical properties of meat proteins involves 3.0 p.m.y.
- (b) Chemistry of meat fats and fat-protein interactions involves 3.5 p.m.y. at Beltsville. A research contract at Florida State University, Tallahassee, involves 0.3 p.m.y. in a study of the interactions of meat pigments with tissue lipids with the objective of developing methods to control rancidity and pigment fading.
- (c) Study of improved meat products and processing methods involves 2.5 p.m.y. at Beltsville; a research contract at Michigan State University. East Lansing, involves 0.2 p.m.y. in a study of the

utilization of dry milk solids in sausages. In addition, research sponsored under P. L. 480 grants is under way at:

- (1) Ministry of Agriculture, Cambridge, England (1.3 p.m.y.), in study of the accelerated freeze-drying of meat.
- (2) Experiment Station for Food-Preserving Industries, Parma, Italy (0.8 p.m.y.) in study of the corning of beef.
- (3) British Food Manufacturing Industries Research Association Surrey, England (0.7 p.m.y.), in study of pigment formation and color fixation during the curing of pork.
- (d) Work on the chemistry of flavor of meat and meat products involves 2.5 p.m.y. at Beltsville.
- (e) Research on the microbiology of meat and meat products involves 3.5 p.m.y. at Beltsville.

In addition, research sponsored under a P. L. 480 grant is under way at the Research Institute of Meat Technology, Finland (0.5 p.m.y.) in study of the influence of microorganisms on flavor development in sausage.

(f) Federal Tesearch on <u>frozen meats and time-temperature</u> tolerance is presently confined to a research contract involving 0.3 p.m.y. at Oklahoma State University, Stillwater, on tenderness reversion in frozen beef.

In addition, research sponsored under a P. L. 480 grant is under way at the Low Temperature Research Station, Cambridge, England (1.0 p.m.y.) on enzymes which attack connective animal tissues, for better understanding of freezer damage in frozen meat.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 25.4 professional man years devoted to meat research and divided as follows: (a) Chemistry and physical properties of meat proteins, 6.8; (b) chemistry of meat fats and fat-protein interactions, 1.9; (c) improved meat products and processing methods, 8.3; (d) Chemistry of flavor of meat and meat products, 3.5; (e) microbiology of meat and meat products, 1.9; (f) Frozen meats: time, temperature, tolerance, 3.0. Industry and other organizations are also conducting valuable research programs on meat and meat products.

Most of the research effort of the American meat packing industry is directed to industrial utilization of by-products, but some research is conducted on better quality control devices; machinery

and automation for packaging, sausage making, variety and luncheon meat handling, and canned meats; improved formulations for canned comminuted meats; development of new products; use of tenderizing agents; studies on new curing and smoking methods; improvements in plant layout; and development of new slaughtering equipment and techniques. This program differs from the USDA program in that there is strong emphasis placed on development, and the solving of immediate problems, and comparatively little on basic research. Estimated annual expenditures for research on meat are equivalent to about 100 professional man-years. About 80 per cent of this effort is expended by about ten or a dozen of the larger firms.

There are hundreds of independent laboratories in the United States, and many work, at times, on small, short-term problems for clients in the meat industry. These problems can generally be classed as "fire fighting jobs". A few larger independent laboratories are more or less continuously engaged in some special development problems for the industry. Their work includes problems on sausage formulation, meat product additives, meat microbiology, by-product utilization, meat flavor studies, and package and machinery development. Some of the large foundations have similar interests, including one that works entirely in the meat field. The foundations have programs relating to nutritive values of meat, meat hygiene, meat product development, meat composition, and some fundamental studies. Estimated annual expenditures are equivalent to about 30 professional man-years.

Large food processing companies which are not primarily meat packers carry on some research on new product development such as dehydrated and/or frozen meat products, combination meat dishes, and meat canning. Estimated annual expenditures are equivalent to about 10 professional man-years.

The pharmaceutical industry carries on research in this field from two standpoints. One deals with the extraction of biologically active substances from meat by-products, such as hormones from glands. The other deals with the development of agents, such as antibiotics, for use in meat processing. By and large, the latter effort has been unsuccessful but remains active. Estimated annual expenditures are equivalent to about 20 professional man-years.

Chemical companies and equipment manufacturers are engaged in the development of new processing equipment, automation of processing, new packaging materials, development of chemicals as additives, new sanitizing agents for plant use, and development of special chemical by-products. Estimated annual expenditures are equivalent to about 10 professional man-years.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A major protein in meat is myosin; its properties and composition can be readily changed by a number of factors. Two types of conformational changes have been detected; in the presence of lithium bromide or potassium iodoacetate the diffusion constant increased while the sedimentation rate decreased; although the myosin particles became less elongated, the molecular weight did not change. During storage myosin undergoes two simultaneous molecular changes: a portion disaggregates into a smaller molecule while other portions aggregate into two larger particles, probably a dimer and trimer.

Factors affecting the extraction of meat protein components have been investigated thoroughly. In sample preparation, grinding the meat was sufficient for general extraction. In the centrifugation step packing of the sediment gave an increase in total yield of protein as the rate of centrifugation increased. At least 85% of the soluble protein could be obtained with one extraction. Yield of extractable proteins depended upon salt concentration and hydrogen ion concentration. Over a wide span of pH and salt concentration swelling of ground meat was directly related to its content of salt-soluble and salt-insoluble proteins, but not that of the water-soluble proteins.

The protein extraction studies described above have been carried out using seven different muscles from the same carcass. The trend with pH was similar in all cases, but there were large differences in the values obtained from the different muscles. Variations in these properties were also measured on samples taken from one muscle at five intervals during aging for twenty-eight days. Marked changes were found in both magnitude and pH effect on the extraction of non-protein nitrogen and protein, and on the water retention of the residues.

To obtain basic information about the relation between tenderization of meat and proteolysis of the meat proteins by tenderizing enzymes a new physico-chemical method of analysis was developed. This method depends upon changes in the structure of meat when proteolytic enzymes split a few peptide bonds in the meat protein. In general, above pH 7 the initial rate of proteolysis increases in inverse proportion to the concentration of hydrogen ions. Also, 0.6 molar potassium chloride increased the reaction rate about 50%. Comparative studies with trypsin, chymotrypsin, papain, and a commercial tenderizer product were also made. Similar measurements, using myosin instead of beef muscle, gave results of a similar nature. Proteolytic enzymes find wide use in tenderizing meat and meat products.

Current methods measuring tenderization involve panel studies, mechanical devices or histological techniques that require large samples, or long time periods, or both. Only the histological techniques give any information about how the meat structure is modified by tenderization.

The moisture retaining characteristics of protein in meat processing, and an understanding of the variation of these characteristics with respect to ionic strength, pH, and inherent differences among the several fibrillar proteins, are essential to improving existing processing techniques and devising new ones. Better methodology for studying water holding is considered of primary importance in the industry to the improvement of many process control methods.

B. Chemistry of meat fats; fat-protein interactions Organic compounds containing the carbonyl group are responsible for many of the undesirable flavors in stored meats and meat products. Presumably these objectionable substances arise from fat through a series of reactions which require oxygen at least as an initiator. Although this reaction can be delayed in some cases by the use of antioxidants, development of adequate protective measures awaits a better knowledge of all the products formed in these oxidation reactions and of the mechanisms by which they are formed. To date research on this project has developed improved methods of analysis for the complex mixtures of carbonyl compounds which result from the oxidation of fat. The more potent flavor producing compounds are relatively unstable and reaction conditions which give quantitative yields of derivative with one type of carbonyl compound may give low yields with another type, or even transform some into something else with entirely different flavor characteristics. These studies with such a mixture of compounds. present in meat or meat fat in only trace amounts, sometimes parts per billion are time consuming; but real progress is being made.

To speed up the fat oxidation process in the laboratory an inert powdered material, as celite or silica gel, has been coated with films of fat to which various potential promoters or inhibitors were added. Preliminary results with salt, lipoxidase and tocopherols showed differences in the pattern of carbonyl compounds formed. This project is developing fundamental information which has tremendous value in clarifying the mechanisms of oxidation or rancidification. Lipid oxidation deteriorates product quality, or at the very least, limits storage of many meat items, particularly such items as frozen meat, precooked meat dishes, and cured meat products, and thus is a problem of world wide importance and interest. Determination of the types of reactions involved affords a basis for developing protection against oxidative deterioration.

By contract with Florida State University a connection of meat pigments with lipid oxidation has been established. Cooking partially destroys the pigments and at the same time produces substances which tend to prevent lipid oxidation. Preliminary results indicate that both the antioxidant BHA and a tripolyphosphate ascorbate mixture protect cured meats from oxidation during freezer storage.

C. Improved meat products and processing methods
Addition of 3.5% nonfat dry milk to comminuted meat products
increased yield and tensile strength, and had no effect on flavor or
color; 10% added milk solids also gave an acceptable product. Use
of fermented milk solids (dry whey) introduced a tangy flavor; nonfat dry milk also had to be added to achieve the needed water binding capacity. Adoption of these surplus foods as ingredients in
sausage-like products could result in a significant increased use
for them.

Increased fundamental knowledge of the chemical reactions involved in meat curing is essential to improvements in this type of product. Since the binding of electrolytes and water, and emulsification of fat, by meat proteins are recognized as essential reactions in acceptable comminuted meat products, studies were initiated to determine the effect of hydrogen ion and salt concentration upon this binding power. Centrifugation at 144,000 times gravity for 24 hours precipitated 99.5% of the soluble proteins. Binding capacity of salt soluble proteins was not affected by change in salt concentration at pH values over 6. Use of a technique devised for determining the emulsification capacity of tissue containing different amounts of "added water" showed that reduction in "added water" reduced the capacity of tissue to emulsify fat. Centrifugal fractionation of muscle suspensions in sucrose solutions and subsequent analysis showed the distribution of zinc in five fractions to be: filtrate, 1.23; structural tissue, 3.47; nuclei, 0.90; nitochondria, 0.06; and microsomes 0.05 milligrams zinc per 100 grams of sample.

The biochemistry of pork muscle pigments is the subject of a P. L. 480 grant to the British Food Manufacturing Industries Research Association. The meat pigment in oxidized form has been isolated in 70% purity. It forms a new pigment complex with nitric oxide, a gas which can be formed from reaction of the nitrite in curing agents with a reducing system in pork flesh. This new substance resembles the normal cured meat pigment, but differs in behavior, decomposing at once in contact with air. The rapid fading sometimes observed in hams after cutting might be associated with the presence of this pigment.

D. Chemistry of flavor of meat and meat products
Changes in the natural flavor of meat can be influenced by chemical reactions which occur normally during processing and storage, or by the use of properly selected microorganisms which result in formation or intensification of desirable flavor characteristics. Cured meats involve the addition of one or more salts which have an effect on growth of microorganisms; obviously if changes in flavor of cured meats are to be produced with microorganisms, strains must be selected which grow in the presence of varying concentrations of salts. Studies have been made with a large number of salt tolerant bacteria at salt concentrations varying from 0.5% to 12% sodium chloride.

The successful development of an inoculum for ham brines could have a profound effect on the acceptability of a major agricultural product which has been slowly losing its preferred status in the American diet. Better knowledge of the physiology of halophilic microorganisms is essential to such an achievement. In studies with 69 cultures differences in morphology at the two salt concentrations were recorded. Enzymes inhibited or not produced in media containing 12% sodium chloride were lipase and gelatinase and some of the enzymes involved in cleavage of sugars.

The important role of fats in meat flavor is recognized. Evidence collected in this research shows that phospholipids, one of the components of the fat fraction, do not contribute to desirable meat flavor, but that they may contribute to poor flavor.

One reason for restricted utilization of lamb and mutton is an objection that many consumers express for lamb flavor. A distinctly unpleasant volatile fraction has been isolated from lamb fat; elucidation of its chemical nature may lead to prevention of its formation or modification of its effect. In lamb flavor studies the lean portion of the meat has the same basic meaty aroma found to be characteristic of pork and beef. A fraction was isolated from lamb fat with a potent "muttony" flavor that was almost carbonyl free; thus, it is believed that both fat and water soluble components of the meat contribute to this flavor.

In these studies on flavor composition of meat extracts and volatiles important improvements have been made in analytical procedures, particularly in gas chromatographic techniques.

E. Microbiology of meat and meat products
Investigations on microbial activity at low temperatures involve a twofold approach. One is a study of the growth of the microorganisms. One of the enzyme systems that is active at temperatures considerably below that at which the microorganism will grow is the lipase, or fat-splitting enzyme.

By the use of known, mixed triglycerides the high degree of specificity of lipases from psychrophilic bacteria for the fatty acids in the 1-position has been confirmed. These enzymes were also found to be active at 0°F., the minimum temperature at which frozen foods are usually stored. Some activity was still evident within three weeks at -30°F. Analysis by gas chromatography of the fatty acids released showed a definite shift toward the unsaturated fatty acids as the temperature decreased.

Studies have also been made of the activity of lipases from other bacteria, yeast and molds. These microorganisms have been shown to vary considerably in the type of fatty acids split off from natural fats. Certain ones appear to have a greater affinity for palmitic and stearic acids while others more readily attack the unsaturated acids. The lipases from all microorganisms examined were active in frozen substrates even though some of the organisms would not grow below 50°F.

In order to study the nutritional requirements for lipase formation, a synthetic medium has been developed in which members of the genus Pseudomonas will grow and elaborate enzymes. Studies with thick-cell suspensions have permitted the investigation of an extracellular enzyme without the added complication of cell multiplication. This differentiation has indicated that the enzyme-synthesizing mechanism ("template") is present in the cell regardless of the conditions of cell growth. The functioning of this mechanism, however, is dependent upon the nutritional and physical conditions of the environment in which the cells are held. Thus, a food product might be held under conditions favoring bacterial growth but not lipase production and under subsequent conditions the lipase could be elaborated by preformed cells.

# F. Frozen meats: time temperature tolerance

Studies conducted at Beltsville some years ago indicated that freezing had a marked tenderizing effect on beef steaks when they were frozen rapidly at low temperatures (-50° to -70°F.), thawed, cooked, and tested. When the steaks were stored in the freezer, as would be the case in practice, the initial gain in tenderness was soon lost. Further confirmation and explanation of this pehnomenon, and the development of means to prevent its occurrence, are the subject of a contract with Oklahoma State University.

Statistical analysis of taste panel ratings for tenderness and shear force values revealed no significant difference in tenderness between the unfrozen control steaks and those frozen and stored for the various times and temperatures indicated. Nevertheless, shear values were altered by the treatment stimuli. Steaks frozen at +15°F. showed an increase in shear values over the unfrozen control

steaks. This decline in tenderness became more pronounced as storage time was extended. Freezing at temperatures of 0°F. and below, however, produced a decrease in shear values of the experimental steaks. The data suggested that a part of this increased tenderness was forfeited during freezer storage; yet the samples frozen and stored for 12 weeks still sheared lower than their unfrozen controls. Decreasing freezer temperatures below 0°F. produced no consistent increase in tenderness.

Panel scores for flavor and overall rating indicated a preference for the frozen and stored product, whereas the juiciness values showed a significant decrease with storage time.

Through a P. L. 480 grant to the Low Temperature Research Station in Cambridge, England, the nature of connective tissue structure is being investigated, particularly the structure of the chondroitin sulphate protein complex. This complex consists of a central protein core around which are arranged many chondroitin sulphate chains in a structure resembling a spiral brush. The protein core carries a second polysaccharide, containing galactose and glucosamine, and it remains to discover how this polysaccharide is attached and the nature of the links between the chondroitin sulphate chains and the protein core. As would be expected by its structure, both hyaluronidase and several proteolytic enzymes attack the complex, but they degrade it in different ways. In the work on elastin it has been discovered that in some tissues the fibrous protein is intimately associated with a second system of proteins which contain sialic acid, hexosamine and neutral sugars and which appear to be combined with lipid in the native tissue. Some progress has been made toward isolating and separating this new family of mucoproteins, and it is believed that they are the substrates for the enzyme elastomucase which has a strong synergistic effect on the action of pancreatic elastase.

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<sup>\*</sup> Work done under a P. L. 480 foreign research grant.

ANIMAL FATS AND OILS - INDUSTRIAL UTILIZATION
Eastern Utilization Research and Development Division, ARS

<u>Problem.</u> The 3.5 billion-pound-per-year output of inedible fats is one of the major products of the livestock industry. It also is one of major concern, because while production of animal fats has doubled in the last 15 years, its principal outlet (in soap) has declined sharply, and is still declining.

The best answer to the question of what to do with huge amount of fats is to find new uses through utilization research. Already utilization research has played a leading role in finding new uses for some 600 million pounds of animal fats, and thus helped retain markets for fats. There is need, however, for new uses not merely to retain or defend markets, but to expand them, and to upgrade the value of animal fats. The organic chemical industry presents a good opportunity for expanded markets, producing as it does a multitude of products—polymers, plasticizers, insecticides, herbicides, lubricants, paper chemicals—totaling 10 billion pounds. Animal fats possess "built-in" properties which make them potentially useful as raw materials to the chemical industry, but research must be done to realize this potential.

Both basic and applied research are needed; the basic to establish the fundamental facts as to composition, separation of constituents, and their chemical activity; the applied to put these facts to work in developing new industrial products.

An increase of 1 cent per pound in the value of animal fats would provide an additional revenue of \$35 million to the livestock industry. This revenue will help the industry and growers in the same way as revenue from other animal products and by-products.

The attainment of an increase in livestock consumption requires both applied and basic research. Applied research is the forerunner of commercial practice and is an indispensable element in successful development. But applied research is based on the foundation of fundamental knowledge that is acquired through basic research, and represents the exploitation of this fundamental knowledge. The supply of fundamental knowledge must be maintained and expanded if applied research is to be most effective and fruitful. The need for basic research has been pointed out by the Commission on Increased Industrial Use of Agricultural Products, by the National Agricultural Research Advisory Committee and by other responsible groups.

#### USDA PROGRAM

The Department has a broad program of basic and applied research at Wyndmoor, Pennsylvania; and at additional locations where contract

research is being carried out involving chemistry and physics, aimed at developing new and improved products from fats for use in industry. Total man-years are 59.5. Of this, 24.0 p.m.y. are devoted to studies on chemical composition and the physical and chemical properties of animal fats at Wyndmoor. Studies involve fatty acid composition of animal fats using the latest techniques in chromatography and other techniques; intra- and intermolecular structure of pure components and derivatives and factors that influence development of off-flavors in fatty foods. A contract at Villanova University, Villanova, Pa., involving 0.3 p.m.y. deals with structural characteristics of purified triglycerides. Contract research on chemical and physical characteristics of organic peroxides involving 0.8 p.m.y. is going forward at the University of Pittsburgh, Pittsburgh, Pa.

Research on improved polymers, plastics, resins and lubricants involving 15.0 p.m.y. at Wyndmoor is conducted in the preparation of new products from fats through vinyl polymerization, condensation polymerization and synthesis of organic compounds for use as plastics, plasticizers and lubricants. Work on preparation of copolymers of octadecyl acrylate was completed during the report period. 5.0 p.m.y. are being devoted at Wyndmoor to research on development of improved synthetic detergents based on animal fats, which includes preparation of \alpha-sulfofatty acids for use in synthetic detergents, and study of soap-detergent combinations for use as bar detergents. 14.0 p.m.y. are being devoted at Wyndmoor to exploratory investigations of new chemical derivatives of animal fats for use as chemical intermediates for industry. A contract with the University of Pennsylvania, Philadelphia, Pa., involving 0.4 p.m.y. provides for determining dielectric properties of longchain fatty acid compounds and heats of combustion of certain fat compounds.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported they were devoting 1.2 p.m.y. to research on chemical composition, physical properties and autoxidation and 0.5 to research on new chemical derivatives.

It is estimated that <u>industry</u> is conducting research on animal fats to the extent of about 180 p.m.y. This is divided among the meat packing and rendering industry, 100 p.m.y. devoted largely to applied research; chemical companies, 50 p.m.y. devoted largely to applied research and development; detergent manufacturers, 10 p.m.y. devoted largely to production problems; research institutes and foundations, 20 p.m.y. in research largely in the field of biochemistry and nutrition.

#### PROGRESS

- A. Chemical Composition and Physical Properties; Autoxidation
- 1. Fractionation and Analysis of Lipids. More exact knowledge of the composition of animal fats, particularly with respect to the less common components, is of paramount importance in their utilization. The development of chromatography and other newer techniques has made it possible to separate many of the components of fats that was not possible by the older chemical methods. In conduct of this work, constant effort is being made to improve the analytical techniques so they might be applied to further separation and determination of fatty constituents.

These advanced analytical techniques have been applied to characterizing the fats in a number of products. Studies of the lipid components of hides involving the stratigraphic analysis of hide substance have shown the presence of unusual fatty acids containing odd-numbered (C<sub>13</sub> and C<sub>23</sub>), low (C<sub>12</sub>, C<sub>11</sub>) and high (C<sub>21</sub>) monounsaturated acids. Wax esters, found chiefly in the outer fayers, consist of sterol esters and esters of long-chain alcohols. (Studies on hydrogenation of fats have shown that certain hydrogenation catalysts did not produce preferential hydrogenation of polyunsaturated acids over the monosaturated acids, nor produce trans double bonds.) In studies on lard composition some 29 fatty acids have been identified as constituents of lard, and quantitative determination of 26 made. Studies on toxic chicken fat showed the presence of large amounts of conjugated linoleic acids compared with trace amounts in non toxic feed. A quantitative estimation of the fatty acid distribution between normal and high stearic acid fed rabbits has been made. A high stearic acid content of the diet was not reflected by a large increase in the stearic acid content of the rabbit fatty tissue. Thin layer chromatography has become an increasingly useful tool for many lipid studies. Improvements made in techniques and accessories for gas-liquid chromatography and thin layer chromatography are important in the further development of these procedures as quantitative methods of analysis of fats and their chemical derivatives.

2. Composition Changes of Animal Fats in Foods. Earlier work carried out on the problem of autoxidation of fats using pure amino acids and fatty compounds in model systems has shown that histidine has a marked catalytic effect on the autoxidation of methyl linoleate at 20°C. With oxygen in the dark. Oxidation values corresponding to 44% oxidation of the ester in the presence of histidine compares with 2.5% conversion when no histidine was present. Other amino acids did not have this effect.

Studies on this problem have been pursued further, determining the effect of other factors such as metal ions, pH, and emulsifiers, on rate of oxidation. The following progress has been made in these studies: (1) histidine is a powerful pro-oxidant for emulsified linoleate but not for dry linoleate, the pro-oxidant effect being enhanced many-fold by the presence of ionic iron, (2) ferrous and ferric ions are more powerful pro-oxidants than cupric ions in emulsified linoleate, either alone or with added histidine, (3) the pH effect is important, maximum pro-oxidant effect occurring at pH 6.5, and (4) the pro-oxidant effect of histidine can be inhibited with phosphate.

Further studies on the effect of the relationship of concentration of histidine to Cu<sup>++</sup> and Fe<sup>++</sup> ions in inhibiting autoxidation of fats reveal a rather complex picture. For example, in a 1:1 molar ratio of histidine to Cu<sup>++</sup>, the histidine increased the pro-oxidant effect of Cu<sup>++</sup> on linoleate emulsions, whereas in molar ratios of 2:1 the reverse effect was noted. In contrast, the pro-oxidant effect of Fe<sup>+++</sup> is enhanced by histidine only when the ratio of histidine to Fe<sup>+++</sup> is relatively high. At low molar ratios, the pro-oxidant action of Fe<sup>+++</sup> is inhibited. These findings might be used to explain the batch-to-batch variation in such fatty foods as whole milk. In such a complex and delicately balanced system as milk, slight variations in the ratios of certain components might either enhance fat stability or decrease it.

The findings that non-ionic emulsifiers and phosphate do not support the pro-oxidant action of iron and histidine in the oxidation of emulsified linoleate may lead to questions on the validity of many previous autoxidation studies in which non-ionic emulsifiers have been used. From the results it would not appear justifiable to apply information obtained with anhydrous fats to preserving fat stability in foods containing water, or to emulsion polymerization of unsaturated organic monomers without first making studies in aqueous systems.

3. Synthesis and Physical Properties of Pure Triglycerides. Investigations on the synthesis and determination of structure and properties of pure triglycerides are being carried out through "in-house" research and through contract research with Villanova University. The "in-house" research has as its objective obtaining basic knowledge of the physical and chemical properties of the individual mixed glycerides of animal fat and how these properties are affected by the degree of unsaturation of the component fatty acids and by the relative position of these fatty acids in the glyceride molecule. The contract research aims to determine the molecular configuration of a simple saturated triglyceride through X-ray diffraction of a single crystal with a view to utilizing the basic information obtained in modifying fats to obtain specific physical characteristics in compositions in which they are employed.

The "in-house" phase of these studies has been completed. Methods have been developed for preparing and characterizing a variety of glycerides. The basic knowledge of the structure and behavior of pure glycerides accumulated under this line of study is necessary in a long range program of research aimed at developing new and improved products from animal fats. This type of investigation is being continued under a new project dealing with the structure of derivatives and components of animal fats employing a diversity of physical instrumentation. Investigations are being carried out on the structural features of closely related esters of fatty acids using low temperature absorption spectroscopy in the infrared region. New and improved methods for structure determination are needed as a basis for research on industrial utilization of chemical derivatives of fatts.

## B. Improved Polymers, Plastics, Resins and Lubricants

- 1. Resins. Two accomplishments have resulted from work carried out on investigations on resinous materials from animal fats: (1) preparation of polymers of viny1 stearate which are potentially useful as synthetic fibers with good water resistance, and (2) a general method has been devised for preparing amides from esters at a low temperature, which has attracted considerable industrial interest. Industrial development of these processes would serve as an outlet for substantial quantities of inedible animal fats.
- 2. Plastics. Since the plastics and plasticizer fields offer large potential outlets for chemical intermediates derived from animal fats, a variety of chemical components and derivatives of animal fats are being investigated for use in these fields.
- (1) Vinyl copolymers from animal fats have been studied under a contract with the University of Illinois, supported in part by EU, and these studies are being continued under a contract with the University of Arizona, also supported in part by EU. Much basic information was developed in this work applicable to the preparation of vinyl polymers from animal fats, which has been reported on in 16 papers and a report on the conversion of agricultural products to polymeric materials, which makes possible the preparation of a wide variety of new copolymers of viny1 monomers with assured consistency of behavior. An extension of the work carried out at the University of Illinois is being carried on at the University of Arizona. Polymers derived from such animal fats derivatives as methyl and vinyl oleate and phenyl stearic acid are being prepared. Studies are also being carried out on the homopolymerization of vinyl dichlorostearate and the copolymerization of vinyl dichlorostearate and vinyl chloride. The copolymers have viscosities ranging from .7 to .9 and softening points between 50 and 100°. Polymers and copolymers of vinyl tetrachlorostearate also have been made. The results of this

work contribute to an assessment of the value of these fat-based monomers in the plastics industry.

- (2) Octadecy1 acrylate polymers have been investigated further. Attempts to improve the impact resistance of copolymers of octadecy1 acrylate by copolymerizing with styrene have resulted in impact resistance equivalent to the best high-impact commercial polystyrene. The octadecy1 acrylate polymers have properties that make them promising for industrial outlet. They are hard, tough, high-melting waxes that might serve as substitutes for expensive imported carnauba wax. Early attempts at copolymerization indicate that modification of commercial polymers with polymerizable amides may result in useful materials. Further studies have been carried out on copolymerization of N-allylstearamide and, respectively, acrylonitrile, vinylidene chloride and vinyl acetate. The preparation of copolymers of N-allylstearamide, a monomer readily prepared from animal fats, opens a new field of application of fat derivatives in polymer work.
- (3) Studies have been continued on the preparation and reactions of glycidyl esters for use in the preparation of plastic resins. Additional diglycidyl esters have been prepared as part of the program to study the relationship between the structure of the ester and the physical properties of the corresponding resin. The high reactivity of glycidyl esters toward a variety of functional groups indicates the preparation of a variety of polymers will be possible. Evaluation of polymers resulting from diglycidyl esters and various acid anhydrides has indicated a number of interesting physical properties which probably can be improved under optimum curing conditions. Diglycidyl esters derived from fats appear promising as monomers for new resins and as comonomers for modification of existing resins. Hydration of the esters indicates that epoxidized esters can be converted to polyhydroxylated esters in high yield. The successful conversion of glycidyl esters to either monoglycerides or diglycerides should prove an inexpensive route to a variety of polyhydroxylated fatty acid esters.
- (4) In studies on the preparation and polymerization of polymerizable amides from animal fats, monomer reactivity ratios of allylstearamide with several commercial comonomers have been determined. Conversion of monomer to polymer shows an unusual high dependence on the catalyst concentration, and the polymer has a typical allylic low molecular weight of 3000 to 5000. The variety of copolymerization parameters for allyl stearamide indicates that some commercially successful copolymer systems might be found.
- (5) Studies of preparation and reactions of organic-inorganic compounds from fats for use as plastic intermediates have been made. Investigations of the reaction variables and purification techniques were made in connection with dichlorocarbene addition and

etherification reactions to fatty compounds. The dichlorocyclopropanes have compatibilities with polymeric silicone oils. The etherification method affords fatty derivatives with added alkoxy, polyethenoxy, and ester functions of interest in several fields of application.

3. Lubricants. The field of lubricants and lubricant adjuncts is a very active one and offers appreciable potential for animal fat-based compounds. Synthesis of potential lubricants and their evaluation as lubricant additives have been carried out. Compounds have been prepared from a series of alkyl esters of 9(10)-bromostearic and dialkyl malonates by means of the malonic ester synthesis. Phosphonates also have been prepared from lard and oleo oil, and polycarboxylic acids and esters by condensing diethyl succinate and carbonyl compounds. Evaluation of the phosphorus-containing compounds shows very promising results. The materials appear to be effective as "anti-wear" and "EP" (extreme pressure) additives for petroleum lubricating oils and di-2-ethylhexyl sebacate lubricant base.

## C. Improved Synthetic Detergents

- 1. Nonionic Derivatives. Surface active agents have been prepared by the reaction of ethylene oxide with 9,10-octadecandiol and with 9,10-dihydroxystearonitrile. The type of usefulness of such surface active agents appears to depend on the number of ethenoxy groups introduced into the molecule, the smaller number producing the better emulsifying properties. Oxyethylated 9,10-octadecanediols containing 4 oxyethyl groups are excellent emulsifying agents; those with 12 were good wetting agents. The nonionic soaps resemble noionics in general. Soap-like characteristics in foaming and detergency were overshadowed by nonionic characteristics. The products are comparable with other nonionics, but foaming, wetting, detergent and emulsifying properties are primarily dependent on the number of oxyethyl groups.
- 2. Soap-Detergent Combinations. Soap-detergent combinations based on animal fats have possibilities in both household and industria. applications. Certain low or controlled sudsing detergents and soap-detergent toilet bars appear to be the only present examples, and a systematic examination should reveal many other types of utilization possibilities. Based on our current investigations, products derived from the  $\alpha$ -sulfonation of stearic, palmitic and pelargonic acids appear to be a promising type that may have detergent, lime soap dispersing, wetting, foam-stabilizing or emulsifying properties, depending on the particular structure. The  $\alpha$ -sulfonation of pelargonic acid and esterification has led to the discovery of a new type of wetting agent. The  $\alpha$ -sulfonation of the lower molecular weight fatty acids of cocoanut oil and of lauric and myristic acids, followed by esterification, also produced efficient wetting agents.

The best of this general class have wetting and foaming properties in hard and soft water equal to or better than the most efficient commerical wetting agents, with the added advantage that they are not easily hydrolyzed in hot acid or alkaline solution. Emphasis in the work has been placed on preparation of new derivatives and types of usefulness in combinations with soaps or other types of industrial surface-active agents. Symmetrical esters are excellent wetting agents. Diesters, diamides,  $\alpha$ -sulfo tallow alcohols, and sodium alkanesulfonates are new types of derivatives that have increased the potential of  $\alpha$ -sulfo tallow acids as products of commercial importance. Future work will be directed to studies of detergent formulations, soap-detergent combinations, and correlation of structure with useful properties.

#### D. New Chemical Derivatives

1. Addition of Nucleophiles. This work has emphasized the direct carboxylation of unsaturated fatty acids, esters and alcohols; the preparation of the dicarboxylic acids; and the controlled preparation of mono- and diesters of carboxystearic acid. The addition of formaldehyde to the double bond of fatty acids produces new types of useful compounds not hitherto available for study. The reactions give high yields and the products should have wide applicability in areas such as polymers, lubricants, plasticizers and functional fluids. The monoesters of carboxystearic acid are low-melting saturated acids hitherto unavailable. The preparation of 1,3-glycol and m-dioxane in high yield by addition of formaldehyde to oleic acid should provide compounds of wide utility as polymers, plasticizers, lubricants and other types of industrial products. Further studies are required on developing effective methods of separating the glycols from the dioxanes, so their physical and chemical characteristics can be determined.

Addition reactions of this type represent a fruitful field of exploratory research, as they offer a means of preparing new compounds not available through other methods of preparation. After preparation, these compounds must be evaluated for specific uses, which is carried out by a physical evaluation group after the compounds are prepared and purified.

- 2. <u>Sulfur Derivatives</u>. Studies on the preparation of sulfur derivatives of fatty components for industrial use have been completed. Synthesis of sulfonic acids with known position has been successfully accomplished through the thiolacetic acid addition—oxidation procedure. These compounds have been difficult to synthesize previously, and they will be evaluated for use in various applications.
- 3. Epoxidized and Hydroxylated Fat Derivatives. Work in this area involves preparation of long-chain products from oxidized (epoxidized

and hydroxylated) fat derivatives for use in polymer modification. Isopropenyl esters of stearic and oleic acids have been prepared by reaction of isopropenyl acetate with the fatty acid in presence of an acid. Further chemical transformation of the esters yields geminal diesters (2,2-distearoyloxy- and 2,2-dioleyloxypropane) and by analogous procedures 1,1-distearoyloxyethane and 1,1-distearoyl-oxycyclohexane. An interesting by-product in some of these reactions is the fatty acid anhydride. This reaction offers an opportunity to obtain some hitherto unavailable anhydrides by reacting fatty acids with isopropenyl acetate. These compounds being prepared are new and in some cases represent a class of compound not available before. When preparation of the series is complete their physical properties will be determined and they will be evaluated for various applications.

Attempts were made to prepare acylons through reductive debromination of a bromoketone acyl. The anticipated compound was not formed, but instead ethyl stearate or hydroxyacetone monostearate was produced depending on reaction conditions. A straight chain vicinal diketone was produced when methyl stearate was condensed with tetramethyl cyclobutanedione.

Studies on use of isopropenyl esters as acylating agents have been initiated. Isopropenyl stearate reacts with succinimide or phthalimide (acid catalyst) to form N-stearoylsuccinimide or N-stearoyl-phthalimide, respectively. Secondary amines can be similarly stearoylated. Hydrogenolysis of N-stearoylsuccinimide under mild conditions yields octadecanal, octadecanol, and regenerates succinimide. Acylation of nitrogen-containing compounds with active hydrogen atoms opens up an entirely new and broad field of fat chemistry. Acylation of barbituric acid and hydantoin derivatives should permit preparation of pharmaceutically useful materials. Acylation of polyhydroxylic substances (cotton, wool, starch, etc.) should yield substances of widespread importance and use.

4. Phosphorus, Sulfur, Oxygen and Nitrogen-Containing Fat Derivatives for Plastic Intermediates. This area of exploratory research deals with the synthesis of phosphorus, sulfur, oxygen and nitrogen-containing compounds from animal fats for use as plastic intermediates. During the reporting period work was carried out on phosphorus and sulfur compounds, as well as peroxides.

Relatively few synthetic compounds containing more than one phosphorus atom have been prepared and methods for preparing them are quite complex. Fat derivatives containing two phosphorus groups were prepared readily by addition of dialkyl phosphonates to diallyl esters of dibasic acids. These derivatives have not been previously reported. They are thermally stable, high-boiling liquid which will be evaluated as functional fluids, as lubricant additives and as

plasticizers. Since only one phosphorus atom in a high molecular weight plasticizer imparts some flameproofing characteristics to the resulting composition, it is hoped that esters with two phosphorus atoms will be outstanding in this regard. The addition of dialkyl phosphonates to linoleates and to unsaturated natural triglycerides such as soybean and cottonseed oils is a marked advance in this type of synthesis, since compounds containing relatively high phosphorus contents can be obtained in good yield from readily available inexpensive intermediates.

Studies have also been carried out on the direct production of peroxyacids from carboxylic acids and hydrogen peroxide in methanesulfonic acid solution. This reaction has been shown to be a fairly general one, and for the first time aromatic peroxyacids can be obtained directly. Studies on the direct synthesis of sulfonic acids from olefins by conversion to thiolacetates and oxidation have been completed. Isomer-free sulfonic acids from aliphatic compounds hitherto have been unavailable, and the availability of such compounds is necessary for determination of their properties and utility for industrial applications.

5. Dielectric Properties of Fatty Peroxides. This research being carried out under a contract project with the University of Pennsylvania has as its purpose studies on dielectric and related properties of fatty peroxides to obtain basic information for use in developing new products from animal fats. Investigations have been carried out in three physico-chemical areas: dielectric properties of t-buty1 peresters; heats of combustion of peracids, t-butyl peresters and their corresponding non-peroxygen acids and esters; and kinetic studies on the decomposition of t-butyl peresters. Collectively, these studies will provide information on the structure of these peroxide derivatives and important thermodynamic data which will serve as aids in the development of reactions for converting peroxides of fats and their derivatives to more useful chemicals. Recent studies under the contract have shown that certain organic peroxides, notably diacyl peroxides and peroxy acids, can be decomposed to obtain useful and difficult to obtain long-chain compounds. Studies have been directed to increased yields of hydroperoxides. which are based on attachment of a group to the alky1 methanesulfonates, which increases their solubility and reactivity.

# PUBLICATIONS AND PATENTS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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HIDES, SKINS AND LEATHER - PROCESSING AND PRODUCTS
Eastern Utilization Research and Development Division, ARS

To maintain the utilization of animal hides and skins at a profitable level there is need to find new products and processes to provide outlets for about 7 million cattlehides that are now available in excess of domestic needs. The foreign markets that currently absorb these surplus hides are also threatened by the increased hide production and decreased per capita use of leather (the principal outlet for hides) that have dislocated U. S. markets and caused prices to drop so precipitously in the last 10 years. meet this problem there is need for upgrading the quality of raw hides and skins, for reducing the costs of producing leather, and for developing products with improved properties. To achieve these objectives research is needed to develop improved curing processes and agents, more effective control measures for (ante mortem) defects such as grubs, brands and parasite damage, and improved methods of take-off. Fundamental research is needed on the composition of hides to provide basic information on the chemical, physical and physicalchemical properties and reactions of collagen and other hide components for use in studies on chemical modification and on the development of new and improved products and processes. Development of new, more rapid and economic processes for curing, handling, unhairing and tanning hides is needed to reduce the cost of producing leather. There is also need for research on the chemical modification of hide proteins to develop leather products with such improved "built-in" properties as increased resistance to wear, scuffing and deterioration from perspiration, enhanced washability, dry-cleanability and improved dyeability; and to improve the physical and chemical properties of gelatin and glue, the important outlets for by-products.

#### USDA PROGRAM

The <u>Department</u> is conducting a broad program of basic and applied research on hides, skins and leather at Wyndmoor, Pennsylvania; Philadelphia, Pennsylvania; Lowell, Massachusetts; Kansas City, Missouri; and Cincinnati, Ohio; which involves chemists, biochemists, microbiologists, and leather technologists.

The Federal scientific effort devoted to the overall program totals 21.3 professional man-years, as follows:

(a) Chemical and physical properties and structure of hides involves 3.0 p.m.y. at Wyndmoor. This research involves exploratory research on the isolation and characterization of cattlehide components and the organization of the components within the hide structure and their relationship to leather properties. A contract with Lowell

Technical Institute Research Foundation at Lowell, Massachusetts, in the amount of 0.3 professional man year deals with the composition and properties of basement membrane of calf skin. A contract with the Franklin Institute, Philadelphia, Pennsylvania, in the amount of 0.7 professional man-year deals with the development of dynamic testing of leather. A contract with the University of Cincinnati, Cincinnati, Ohio, in the amount of 0.3 professional man-year involves basic studies on the noncollagenous proteins of cattlehides. Studies on the stratigraphic composition of hides was completed during the reporting period.

(b) Chemistry of collagen involves 4.0 professional man-years at Wyndmoor, and concerns determination of the physical-chemical properties of collagen, its soluble components and reaction products with modifying chemical agents, to elucidate the nature of the forces that control the stability and reactivity of this natural polymer and the factors responsible for the unique physical properties of leather. A contract with the Midwest Research Institute, Kansas City, Missouri, utilizing 0.5 professional man-year deals with the physical and chemical properties of collagen.

Additional research sponsored by the Department under grants of P.L. 480 funds is in progress at:

- (1) University of Turku, Finland, (0.6 p.m.y.) for research on the fractionation of gelatin and soluble collagen.
- (2) Central Leather Research Institute, Madras, India, (0.3 p.m.y) on the reaction of polyphenolic tanning compounds with hide proteins (collagen).
- (c) Chemical modification of hides involves 6.0 p.m.y. at Wyndmoor. The program is aimed at developing new products with built-in properties tailored to specific end use requirements.
- (d) New and improved processing involves 3.8 professional man-years at Wyndmoor. This research deals with new techniques for unhairing hides and skins, processes for imparting improved resistance of leather to deterioration by heat and perspiration, and new and improved tanning procedures.

A contract with the Lowell Technical Institute Research Foundation, Lowell, Massachusetts, in the amount of 0.3 professional man-year deals with the processing of leather tanned with dialdehyde starch.

Additional research is in progress under grants of P.L. 480 funds to the following foreign institutions:

(1) British Leather Manufacturers Association, Surrey, Great

Britain, (1.0 p.m.y.) for a study of deterioration of leather by sweat, chemicals and heat.

- (2) Experiment Station for Leather Products, Naples, Italy, for research on the cause of "red heat" (0.7 professional manyear) and on improved tanning methods for United States hides (0.8 professional man-year).
- (3) Central Leather Research Institute, Madras, India, (0.2 professional man-year) on the interrelation of hide quality with the rate of tanning and efficiency of tanning.

Studies on development of improved enzyme unhairing treatment for hides and the evaluation of dialdehyde tanned leathers were completed during this reporting period.

(e) Enzymology of hide processing involves 2.0 professional man-years at Wyndmoor, on studies on screening of commercially available enzyme preparations for their depilatory activity on cattle hides and skins.

A contract with the University of Cincinnati, Cincinnati, Ohio, in the amount of 0.5 professional man-year deals with the enzymic process of unhairing hides.

(f) <u>Utilization of animal protein residues</u> involves research in cooperation with the National Renderers Association, who support one professional worker for research on renderers protein residues (meat and bone meal and tankage) to obtain information for use in developing the best values from the residues. U. S. Department of Agriculture participation in the program is 0.2 professional man-year in a supervisory and research leadership capacity.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported no work in this area.

Industry and other organizations are carrying out a valuable research program. It is estimated that 20 professional man-years are expended by a number of chemical companies who specialize in tannery and packing house supplies, for research on developing new tanning agents, unhairing agents, and the various chemicals used in the tanning and finishing of leather. About 10 professional man-years are expended by the tanning industry for developing new processing methods and techniques used in the tanning industry. It is estimated that 10 professional man-years per year are expended by universities and institutes conducting basic research on the composition and physical structure of hides and skins, on the chemistry of collagen, on the utilization of animal by-products and on improving the chrome tanning process. About 5 professional man-years per year are expended by the

meat-packing companies devoted to new and improved processes for handling and curing hides.

It is estimated that 5 professional man-years per year are expended by research and development corporations for research underwritten by segments of the meat-packing and tanning industries. This research includes projects such as developing a solvent tanning process for sole leather; a process for reconstituting hides; a scuff-resistant finish for shoe upper leather and conversion of tannery by-products to a feed stuff supplement.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE RESEARCH

## A. Chemical and physical properties and structure of hides

1. Distribution and properties of hide components. A better understanding of the nature and distribution of hide components is essential to the development of improved methods of processing hides into leather and to the development of new and improved types of leather. Previous studies have shown that the density of the grain layer is less than the middle portion of the hide and contains considerably more void space than the center layers. Studies have also been carried out on stratigraphic distribution of the fatty components through the thickness of the hide which showed a considerable variation in the amount and nature of the fatty components. These results, obtained through use of a microtome, were based on layers which were only a few square centimeters in area. Bandknife splitting has been used to obtain much larger areas of the split hide, which has greatly facilitated the studies. A technique has been worked out for measuring accurately the void spaces in the hide caused by removal of the hair shaft. Results show that hair shaft volume is only a small percent of the total void space.

The studies on stratigraphic composition of hides for the most part have been completed. Determinations have been made of the distribution of dry matter, nitrogen, hydroxyproline, carbohydrates, hexosamine and lipids through the thickness of the hide. A marked variation in lipid content from layer to layer was found. It was also established that there is a lower density in both the grain and flesh regions of hides and that this density difference can be varied by physical treatments. The results indicate the plausibility of producing improved properties in leather by physical treatment.

2. Composition of the basement membrane of hides. Fundamental studies conducted under a research contract by the Lowell Technological Institute on the composition of the basement membrane of hides have shown that the only recognizable constituents present were elastin fibers and reticular-like fibers which appear to be continuous with similar fibers in adjacent tissue, and mucoproteins which were

concentrated in the basement membrane and did not appear to be present to any extent in adjacent tissue. The mucoprotein nature of the matrix explains the effectiveness of proteolytic enzymes as depilatory agents. It appears that the most efficient enzyme would have specificity for the protein component of the mucoprotein fraction of the basement membrane.

Reversible shrinkage in leather. Certain tanning materials impart the important property of reversible shrinkage to leather. For example, an epoxy resin (Epon 562) under certain conditions produces leathers showing reversible shrinkage. This property is important in imparting special properties to leather that could lead to expanding its use in applications such as leather garments and gloves. Studies to elucidate the mechanism of reversible shrinkage have developed information on the nature of the reaction between the epoxy resin and collagen molecules. The results indicate lysine, tyrosine and hydroxylysine have formed a stable combination with the resin. Since these amino acids are not terminal to the collagen molecule, the  $\alpha$ -amino nitrogen could not be the point of attachment. disappearance of certain amino acids from hydrolysates of epoxy-resin treated collagen suggests that they are involved in the reaction. relatively strong chemical bond between the epoxy resin and amino acid is substantiated by the failure to detect the products using the usual tests for amino acids. Since the epoxy resin has 3 reactive groups, the possible complex nature of the derivatives is quite evident. Quantitative studies indicate that the equivalents of permanently attached resin are a simple multiple of the total resin content.

Identification of the structure produced by the reaction of collagen with the epoxy resin should throw considerable light on the mechanism responsible for the phenomenon of reversible shrinkage. An understanding of the true nature of the phenomenon might enable production of more economical and practical washable leathers.

4. Dynamic mechanical tester. A dynamic mechanical apparatus for use in evaluating the fundamental physical behavior of hide and leather is a valuable tool for obtaining basic information essential to progress in better utilization of hide substance. A research contract with the Franklin Institute of Philadelphia to design and construct an apparatus of this kind has been made, and work is under way. Good progress has been made, and the system proposed by the contractor appears to be highly practical.

# B. Chemistry of collagen

1. Effect of electrolytes on collagen and its solubilization. Studies on collagen being carried out concern the determination of its chemical and physical behavior and the effect of electrolytes on its properties. It has been demonstrated that calcium ions are preferentially absorbed by collagen. A new form of collagen was

also produced to demonstrate that the structure of the collagen fiber depends on its previous environment. Preparations of soluble collagen have been made in a variety of buffer (salt) solutions and studied by means of ultracentrifugation and electrophoresis. Preparations made below a critical pH showed a single sharp sedimentation peak. Preparations of soluble collagen made with phosphate buffers were assayed to determine bound phosphate, and equilibrium-dialysis studies carried out using hide that had been subjected to washing prior to solubilization of the collagen. This was intended to remove polysaccharide hide components. The results showed that the presence of polysaccharides has no effect on the results. The fundamental information resulting from these studies will provide a basis for further studies on the solubilization, modification and stabilization of hide components.

In contract research at the Midwest Research Institute, an interesting and possibly quite significant new method for dispersing collagen was discovered. By forming the hypochlorite or hypobromite, in the absence of excess acid, the derivative disperses completely to give a gel containing as much as 3% collagen. Some degradation takes place, but collagen fibers can be regenerated.

Fundamental studies on the fractionation of gelatin and soluble collagen proteins and characterization of the fractions by chemical and physico-chemical means to provide information for expanding the utilization of hides and skins are being conducted under a P.L. 480 grant at the University of Turku, Turku, Finland. The grantee has investigated the fractionation of gelatins, using ion-exchange methods, and some of the techniques explored appear worthy of further study. These are being applied to a number of samples supplied him by the Eastern Utilization Research and Development Division of the Agricultural Research Service. Studies on the thermal and mechanical properties of rat tail collagen have revealed that the form of the tension-time curve of tendon fibers is changed within a few minutes after its extraction from the tail, and there are indications that the mechanical properties are influenced by the physiological states of the rat. The increased solubility and decreased tensile strength of rat tail collagen do not appear to depend on the amount of carbohydrate tissue. However, carbohydrates could form a protective coating on the surface of the fibers. Differences in the physical and chemical properties are being characterized by X-ray diffraction, infrared spectra, water vapor sorption analysis, binding power for chromium salts, rate of gelation and properties of the derived gelatins. The experimental design is such that the studies can be extended to reveal the tensile strength of recently formed interwoven collagen as a function of associated tissue components. The fundamental information being developed in this work should be helpful to a better understanding of the structure and behavior of collagen, which may have application to developing

improved leather and hide products.

## C. Chemical modification of hides

Improved water repellency for leather. Imparting better water resistance to leather is important in improving its competitive position with respect to leather substitutes. Previous work has shown that silicone is effective for this purpose and that a siliconetung oil mixture is essentially as effective as tung oil alone, which would reduce the cost of the process. Further studies confirm this water-proofing effect. However, the water-proofing agent is incompatible with conventional lubricating agents such as fat liquors, and studies being carried out to overcome this difficulty appear promising. Use of alkenyl succinic anhydride (Casyl B18) dissolved in mineral spirits as the lubricating agent is effective for glutaraldehyde-retanned chrome leather. A low level of water repellent treatment appears to be quite effective and offers promise for an economical use of silicone. The finding that retanning chrome leather with glutaraldehyde greatly simplifies the lubrication step has improved the potentialities for the tung-silicone complex as an economical means of imparting water resistance to leather. Waterrepellency tests are being conducted with full sides of chrometanned splits which were retanned with glutaraldehyde and fatliquored with Casyl B18.

Studies have been carried out on the use of urethane elastomers as impregnants for leather, especially chrome splits, in order to improve the resistance to water and perhaps to abrasion as well. Finished and rough sole leather and vegetable-retanned chrome splits were impregnated with urethanes, with and without a catalyst, in varying concentrations. Water resistance (static) was improved. Finished sole leather showed little change in abrasion, whereas rough sole leather and retanned chrome splits showed an increase in resistance to abrasion. Leathers impregnated with urethanes without catalyst were not as stiff and brittle as leather impregnated with urethanes with catalyst, and showed only slight differences in resistance to abrasion.

2. Chemical modification with dialdehydes. Dialdehydes have been shown to be versatile tanning agents and impart important properties to leather. Glutaraldehyde has been shown to be a good tanning agent for light leathers, either alone or as a combination tannage with chrome. Further studies have been carried out on the application of glutaraldehyde and dialdehyde starch as tanning agents. Studies on the rate of tanning with aldehydes and chrome used simultaneously were carried out. Comparison was made between formaldehyde and glutaraldehyde in such a combination. Both formaldehyde and glutaraldehyde appear to act independently of the chrome.

Skins tanned with glutaraldehyde and chrome were processed into finished garment leather in a commercial tannery. The finished leathers were of good quality. Tests showed a good degree of washability and resistance to a synthetic perspiration. Hot soap solution (wash test) lowered the shrinkage temperature of these leathers by 4 to 10°C. as compared with 22°C. for leather tanned with chrome alone. The glutaraldehyde-chrome leathers were resistant to area change embrittlement when heated in a synthetic perspiration in marked contrast to the behavior of chrome leather. It appears that a relatively small amount of glutaraldehyde is effective in producing these desirable properties in chrome leather.

Cooperative tests with tanners are being carried out to evaluate glutaraldehyde as well as dialdehyde starch in various combination tannages of grain split cowhide intended for upholstery and garment leather. The retention in leather of the desirable properties conferred by glutaraldehyde even when used simultaneously in the same bath with chrome is a step forward toward simplication of the process. This will give it the versatility necessary for ready adoption by tanners. Furthermore, the desirable properties of glutaraldehyde leathers can be conferred on the regular commercial chrome leather by merely adding a small percentage of glutaraldehyde to the normal tanning process. The versatility of glutaraldehyde allows its use along with chrome in the tanning of leather. The development of mellowness without loss of tightness has proved a most valuable property for the production of leathers for the currently popular softee and casual markets. Such leathers may be the answer to the threat posed by sneakers to the shoe upper leather market.

In contract research carried out by the Lowell Technological Institute on production of light leather with dialdehyde starch it was found that the use of ammonia during tanning with dialdehyde starch offers promise of developing suitable posttanning processes for leather tanned with dialdehyde starch along. A satisfactory procedure has been developed for tanning garment type leather. This involves pretanning the skin with a small amount of chrome (2%) followed by retanning with dialdehyde starch. These leathers are almost white and can be readily rewet for staking. The staked leathers are smooth-grained and soft.

# D. New and improved processing

1. <u>Imparting improved deterioration resistance to leather</u>. Leather with greater durability is desired for most applications. Perspiration resistance is one of the properties that needs to be improved. Department research in the past has developed greater perspiration resistance to insole leather. Present efforts are being directed to improving light leathers in this respect, such as glove, garment and shoe upper leathers.

Work has been continued on the use of dialdehyde starch (DAS), glutaraldehyde and tetrakis-(hydroxymethyl) phosphonium chloride (THPC) in various combinations with conventional tanning agents to improve perspiration resistance. Vegetable-tanned bellies retanned with a small amount of glutaraldehyde resulted in improved perspiration resistance. Pretanning sole leather with dialdehyde starch improves perspiration resistance. However, to take advantage of dialdehyde starch, the whole tanning procedure would have to be changed. It was found that dialdehyde starch can be used effectively by incorporating it in the oil wheel, which is the last step in processing. This permits treatment with dialdehyde starch of only selected lots as needed. Another process for conferring perspiration resistance is use of tetrakis-(hydroxymethyl) phosphonium chloride-resorcinol in combination with dialdehyde starch or glutaraldehyde. Skins tanned with tetrakis-(hydroxymethyl) phosphonium chloride-resorcinol and retanned with various commercial tanning materials and then processed into garment suede or grain leathers were found generally to be of acceptable commercial quality.

A fundamental study of the mechanism of deterioration of leather to obtain basic information for use in developing leathers more stable to the action of chemicals, sweat and heat has been in progress at the British Leather Manufacturers Research Association at Milton Park, Egham, Surrey, England, under a P.L. 480 grant. Results obtained show that lactic acid in perspiration is the main cause of damage to chrome leathers and that small amounts of vegetable tannins increased the stability of chrome leather to perspiration. have also been made on the combined action of heat and moisture as a cause of leather deterioration. It was found that chrome-tanned leathers are likely to be more resistant, while semi-chrome and chrome retan leathers are generally badly damaged by exposure to moist heat. Results of studies to determine the mechanism of deterioration suggest that the polypeptides of the chains of the collagen are broken down to smaller units and there is general disorganization of the protein structure. The changes resulting from Y-irradiation of collagen are of a similar pattern, and this may throw light on the process involved.

Studies on stabilization of leather through cross-linking show that tanning by the introduction of cross links, such as is imparted by glutaraldehyde, reduces breakdown of the polypeptide chains and thus tends to stabilize the leather. This confirms observations made in the United States Department of Agriculture that dialdehydes, and in particular glutaraldehyde, are good cross-linking agents and tend to stabilize leather.

2. Enzymatic unhairing of hides and skins. The development of an economical process for enzyme unhairing of hides that would produce high quality leather would have a number of advantages, including

that of stream pollution abatement. When tanned by usual processes for shoe upper leather, the leather from enzyme-unhaired hides is flat, firm and tinny. Tests have been carried out with the object of producing commercially acceptable chrome-tanned leather from enzyme-unhaired stock. Postliming of hides for a day after unhairing produces leather of acceptable quality. This indicates that some physico-chemical change is required in the collagen fibers.

In contract research conducted at the University of Cincinnati, steer-hides unhaired by two different enzyme preparations were vegetable tanned in the usual rocker yard and processed into finished leather. the finished leather from the enzyme-unhaired stock could not be distinguished from the matched sides which were unhaired with lime-sulfide. Appearance and properties were not significantly different.

Production of vegetable-tanned sole leather from enzyme-unhaired hides without need for modifying the tanning process is encouraging. Production of acceptable chrome-tanned upper shoe leather has proven to be much more difficult. However, several facts have been found that may prove to be clues to a solution of the problem.

- New tanning processes. Studies initiated recently are aimed at improving the quality of leather utilizing newly discovered tanning agents. Tannins being investigated include glutaraldehyde alone, aldocryl alone, combinations of these tannins with chrome, formaldehyde and acrolein. Properties of particular interest are finish and durability of shoe upper, suede, garment and other types of leather. Leathers with greater perspiration resistance and durability would be advantageous, particularly for shoe upper leather and work glove leather. Evidence has been obtained that glutaraldehyde increases the perspiration resistance of leather. Findings that use of glutaraldehyde for tanning cattlehides either alone or in combination with chrome is entirely practical from a tannery standpoint have stimulated several tanners to evaluate this tannage in their production. This development is most timely, since it will enable tanners to produce leathers needed for the casual and softee shoe trade.
- 4. Investigations on "red heat." A fundamental study of "red heat" of cattlehides to obtain basic information for use in developing means of preventing this defect in exported American hides has been initiated by the Stazione Sperimentale per l'Industria delle Pelli e delle Materje Concianti, Naples, Italy, under a P.L. 480 grant. Studies are directed to isolating and culturing microorganisms associated with red heat in hides. Over 200 strains were cultured and characteristics determined. Investigations are also being carried out on the extent of damage done to hides by growth of pigmented halophilic microorganisms and the use of chemical agents for inhibiting their development. This information will be helpful

for preventing or controlling damage to hides caused by microorganisms during storage or transoceanic shipment.

5. Conversion of U. S. cattlehides into sole leather in Italian tanneries. Due to difficulties encountered in the conversion of U. S. cattlehides to high quality sole leather in Italian tanneries, research has been undertaken by the Stazione Sperimentale per l'Industria delle Pelli e delle Materie Concianti, Naples, Italy, into the cause of this behavior of American hides as compared with hides from other countries. They have found that American hides possess 10 to 20 times more fat than their domestic hides, and even after degreasing they still had 2 to 4 times as much.

Tanning tests on American hides tanned by Italian processes have been carried out and the leather compared with sole leather produced by using the English process. The grantee has completed the evaluation of U. S. Packer hides obtained from 13 sources. The tests confirmed previous results that U. S. hides have too many defects, such as fat, manure, dirt, grub holes and brands. On the basis of the pilot tanning tests, the nature of the problem of utilizing U. S. Packer hides in Italy and other European countries and the course of the investigation have been defined quite clearly.

## E. Enzymology of hide processing

- 1. Screening commercial enzyme preparations for depilatory activity. Over 50 commercially available enzyme preparations have been screened for their depilatory activity in connection with enzymic removal of hair from hides. Several preparations of bacterial origin and one from a Streptomyces were found to be most active. The hair loosening activity was found to be associated with the proteolytic activity of the enzymes as demonstrated by action on casein, gelatin and elastin. Contrary to reports in the literature, no correlation was found between the amylolytic or elastolytic and depilatory activities of the enzyme preparations. The work carried out in this field has stimulated the interest of many tanners, packers and enzyme manufacturers to investigate commercial possibilities. No serious difficulties have been encountered in the use of conventional tannery equipment such as paddles and drums for unhairing operations. Studies on the screening of enzyme preparations have been completed.
- 2. Properties of leather made from enzyme unhaired hides. Contract research at the University of Cincinnati on the properties of enzyme-unhaired vegetable-tanned sole leather as compared with similar leather prepared from lime-sulfide unhaired hides has shown that finished leather from enzyme-unhaired stock was quite comparable to that prepared from lime-sulfide unhaired hides. Production of acceptable chrome-tanned shoe upper leather has proven

to be much more difficult. While the research has shown that the strength of enzyme-unhaired chrome-tanned upper leather is equal to that of similar leather made from hides unhaired by lime-sulfide treatment, the leather from the enzyme-unhaired hides was firmer and lacked the stretch characteristics required of shoe upper leather. The contractors feel that the firm tinny condition is a result of the nonuniform deposition of the chrome in the fiber. As a result of further systematic exploration of the problem, the number of variables that affect the mellowness and smoothness of grain in enzyme-unhaired stock is being reduced. The pickling process and the type of retannage appear to be most critical. Conditions have been found that produce the desired properties in laboratory size specimens. These should be confirmed using full sides.

# F. Utilization of animal protein residues

Work on this project is being conducted through a fellowship arrangement with the National Renderes Association. The research was reactivated about a year ago when a new senior fellow, Dr. Gorbunoff-Timasheff, was appointed to continue the work. She will be assisted by a junior scientist. Work under the fellowship has been suspended for approximately two years because of resignation of both the former senior and junior fellows.

Work was resumed on amino-acid composition and fractionation studies of a number of samples of commercial meat meal. The meals were fractionated into homogeneous components suitable for further study. A method has been developed for separating the bone from the meat portion of the meal. Studies also indicate a considerable breakdown of the protein of the meat portion of the meal, which evidently takes place in the rendering. It is not known whether this breakdown of the protein affects its feed value. Studies being conducted by the National Renderers Association at Battelle Institute indicate that an enzymic rendering process they have developed may be more economical than the present one. This may produce meal with considerably less decomposition of some of the essential amino acids. The product made by this process would be completely soluble.

The fractionation process developed for separating the bone and meat fractions of meal will permit studies of the isolated protein without complication of mineral constituents. Information being obtained could lead to production of a more uniform product and thus help to stabilize the utilization of this valuable by-product of the rendering industry, which amounts to about 1,300,000 tons annually.

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\* Work done under a P.L. 480 foreign research grant.

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\* Work done under a P.L. 480 foreign research grant.

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#### III. MARKETING RESEARCH

LIVESTOCK AND MEAT - MARKET QUALITY Market Quality Research Division, AMS

Problem. Meat is a very perishable commodity which varies greatly in quality characteristics such as tenderness, juiciness, flavor, and fat content. To insure more uniform grades and standardized products, better objective tests for measuring the quality attributes of meat are needed. Also needed are more effective methods to minimize shrinkage while maintaining optimum quality, bloom, and shelf-life of the product as it moves through market channels.

#### USDA PROGRAM

This work is being conducted at Beltsville, Maryland, with the cooperation of the Animal Husbandry Research Division, ARS, and also in part by research contract with the Universities of Wisconsin and Oklahoma. Research, basic and applied, includes the development of objective methods for evaluating the composition of livestock, carcasses, and meat cuts; the application of ultrasonic techniques to estimate the thickness of backfat and muscling in live hogs, cattle, and sheep, and the use of measurements of the low-level natural gamma-ray emission of meat cuts for estimating their lean content. New techniques for measuring meat tenderness are being developed and evaluated.

The Federal scientific effort devoted to research in this area totals 3.6 professional man-years of which 2.4 man-years is by research contract. The total effort is devoted to objective measurement and evaluation of quality. During the report period, work on criteria for identifying meat-type hogs and feeder pigs (BS 3-74) was completed. Studies on gamma-ray measurements of meat cuts (BS 3-5) were also completed.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 8.1 professional man-years divided among subheadings as follows: Objective measurement and evaluation of quality 6.7, quality maintenance in handling, packaging and storage 1.4. The former includes objective measurement of meat quality on beef, lamb, and pork, including physical and chemical changes in fat and protein; changes in color of different cuts of meat; organoleptic evaluation of tenderness;

histological structure; collagen content; and amino acids; influence of pre-slaughter treatment; effects of marbling, fat covering, color maturity, and other carcass characteristics on the basis palatability components. Under handling, packaging, and storage the work concerns maintenance of product color, control of moisture, prevention of off-odor adsorption, and of microbiological spoilage.

Industry and other organizations also conducted research in this area. The American Meat Institute Foundation research program includes various aspects of meat processing, prepared meats, and meat cookery, and amounts to an estimated annual expenditure equivalent to approximately 3 professional man-years. Many meat packing companies have their own laboratories. However, to a large extent, the work of these laboratories is slanted toward the development of new processing procedures, products, and formulations, thus limiting the research effort on evaluation of market quality and quality maintenance. Much of the research of company laboratories in this area is kept confidential. Estimated annual expenditures are equivalent to approximately 8 professional man-years.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

## A. Objective measurement and evaluation of quality

1. Evaluation of Live Animals in Relation to Composition. Analysis of much of the data from extensive research on the relationship of ultrasonic readings of backfat and muscle thickness of live swine has been completed. Highly significant multiple correlations were found between three or more successive live animal ultrasonic readings of backfat thickness and total separable fat. A highly significant multiple correlation was found between five successive live animal ultrasonic readings of backfat thickness and total lean content.

Research on the use of nondestructive measurements of natural potassium - 40 gamma radiation revealed that natural radioactivity was highly correlated with the percent of separable lean and separable fat of pork hams and beef rounds.

(BS 3-5)

2. Measurement of Tenderness. A new device for evaluating the tenderness of sliced meat has been developed. This Slice-Tenderness Evaluator (STE) was used in conjunction with a standard

commercial testing instrument, in cooperative tests with ARS, to evaluate pork roast slices. The evaluation with the STE were in good agreement with a subjective taste-panel evaluation and a standard objective method which involves the use of meat cores (Warner-Bratzler shear).

(MQ 3-34)

3. Factors Influencing Quality in Pork. A total of 466 carcasses were selected for this study and included animals of known and unknown histories. Marbling in the longissimus dorsi and chronological age were found to be the most important factors associated with palatability. Fresh pork loins were generally acceptable in palatability if they contained approximately 20% of intramuscular fat on the moisture free basis and were from animals less than 220 days of age. The palatability of commercially cured hams was acceptable regardless of chronological age, carcass weight or intramuscular fat content. Bacon became more tender with increasing amounts of intramuscular fat. Flavor and juiciness of bacon were not affected by differences in chronological age, carcass weight, or intramuscular fat. Loin eye area per hundred pounds of carcass weight was of greater value in the prediction equation of lean yield than was unadjusted area of the longissimus dorsi. The gilt carcasses exhibited higher lean cut yields, larger loin eye areas, higher percentages of loin and ham, less backfat thickness and were longer than barrow carcasses.

(MQ 3-9(C))

4. Influence of Bovine Age Upon Meat Characteristics and Grade. The contractor has been evaluating the work of the past 3 years and making statistical analysis of the data. Several publications have already been prepared and several more will be ready in the next few months. Some of the important findings to date are the following: It was found that tenderness of the longissimus dorsi steaks as measured by the Warner-Bratzler Shear and panel (with marbling of each carcass at or closely approaching either the "slight amount" or "slightly abundant" level) decreased significantly with increasing animal age. The greatest difference in tenderness was observed between the 18 and 42 month age groups. The effect of aging the meat 14 days varied with animal age, marbling level, and the tenderness measure used. Moisture, ash, and protein contents of loins were not significantly different for the age groups except that the 6-month old calves had slightly higher moisture values.

(MQ 3-10(C)

5. Criteria for Identifying Meat-Type Hogs and Feeder Pigs. use of the live animal probing technique was demonstrated in purebred herds of swine as a means of assisting breeders in locating and identifying meat animals and strains. In addition, live-hog measurements of 107 crossbred barrows and gilts at 75, 125, and 225 pounds were studied to determine the relative accuracy of backfat probes, scores for market grades, and various body measurements for estimating carcass value. Backfat thickness generally was the most important measure for predicting individual differences in yield of fat cuts, yield of lean cuts, yield of lean meat in ham, and loin-eye area. However, with 75 and 125 pound gilts, score for market grade was slightly more accurate for predicting yield of lean cuts and yield of lean meat in ham than was backfat thickness. The results suggest that of the 12 live-hog measurements used, for 225 pounds swine and lighter weights, backfat thickness was the best single measurement for estimating meatiness in hogs, followed successively by depth of middle, width of middle, width over ham, and score for market grade.

(BS 3-74)

# PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

# Objective Measurement and Evaluation of Quality

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LIVESTOCK, MEAT, AND WOOL - MARKETING FACILITIES, EQUIPMENT, AND METHODS

Transportation and Facilities Research Division, AMS

Problem. Many of the livestock, meat, and wool marketing, slaughter, and warehouse facilities occupied today are obsolete and the work methods that can be used in such facilities are antiquated. consequence, labor costs are excessive and they are increasing. Many firms still are occupying facilities designed primarily for handling rail receipts and shipments even though the majority of these products today are moved by motortruck. This situation also adds to handling costs. Numerous firms are occupying "makeshift" facilities which were designed for other uses or for work methods and operations of a bygone era when labor costs were low. Changes in transportation systems, population growths and shifts, and advancements in technology also have brought about changes in the types of facilities needed - such as the livestock auction markets, commercial feedlots, and hotel supply houses. Most private firms handling livestock, meat, and wool lack the technological and engineering skills necessary to plan and develop suitable facility layouts and designs and to select the types of equipment needed. Therefore, engineering and related research is needed to provide guidelines for industry to increase efficiency; including the designing of improved plant layouts, which will provide proper arrangement of work areas to minimize travel distances and excess handling. and the development of work methods that will permit use of mechanized and automated equipment rather than the relatively high-cost manual methods now used.

### USDA PROGRAM

The Department has a continuing long-term marketing research program involving industrial engineers and agricultural economists engaged in both basic and applied research to develop new and improved work methods, equipment, and facilities for livestock markets, meat wholesalers, and wool warehousemen. Livestock market research is carried on at Washington, D. C. Part of the work in this area is carried out in cooperation with the Toledo Scale Corporation and the Central Missouri Livestock Auction. One project dealing with commercial cattle feedlots was completed and terminated during the year. The research on livestock slaughtering and meat packing wholesaling at Stillwater, Oklahoma is cooperative with the Oklahoma Agricultural Experiment Station. Wool warehouse research is carried on at Washington, D. C.

The Federal effort devoted to research in this area totals 5.3 professional man-years; 2.5 man-years (including 1.4 man-years of contract work) to livestock marketing, 2.0 man-years to meat facilities, 0.3 man-year to wool warehouses, and 0.5 man-year to program leadership.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

Equipment Manufacturers. Several manufacturers of equipment for livestock markets (commercial feedlots, auction markets, and terminal stockyards) are engaged in engineering research to develop improved mechanical devices for performing the physical handling (receiving, feeding, selling, weighing, and loading out) and other related operations required in the marketing of livestock. Estimated expenditures and equivalent to approximately 15 professional man-years annually. A number of companies manufacturing equipment for slaughtering, fabricating, and processing meat and meat products also are engaged in equipment development research. A few of these companies also do service type research to develop layouts for specific work areas in various types of plants for the use of their equipment. Estimated expenditures are equivalent to approximately 150 professional manyears. A few manufacturers of equipment used in receiving, grading, storing, and loading out wool are engaged in engineering research to develop improved equipment for reducing wool warehouse labor requirements. Estimated expenditures are equivalent to approximately 2 manyears annually. The estimated total annual expenditures for livestock, meat, and wool engineering and equipment development research are approximately 167 professional man-years.

Livestock Market Operators, Wholesale Meat Distributors, Meat Packers, and Wool Warehousemen. Although livestock market operators do not have organized engineering research staffs, individual market operators occasionally conduct engineering research on specific problems for short durations. The estimated expenditures are equivalent to 2 man years annually. A number of slaughterers, packers, processors, and fabricators of meat and meat products have organized engineering research staffs to develop improved work methods and plant layouts. Estimated expenditures are equivalent to approximately 75 man-years annually. Wool warehousemen do not have organized engineering research staffs but individual warehouse operators occasionally conduct engineering research to develop specific items of equipment. The estimated expenditures are equivalent to 2 man-years annually. The estimated total expenditures of these industry groups are approximately 79 professional man-years annually.

#### REPORT OF PROGRESS OF USDA AND COOPERATIVE PROGRAMS

# A. Layouts and Work Methods for Commercial Cattle Feedlots

At Washington, D. C. a manuscript entitled "Improved Methods and Facilities for Commercial Cattle Feedlots" was completed and published. The report shows improved layouts for commercial cattle feedlots having capacities respectively for 1,000 5,000, and 10,000 head. The layouts and work methods proposed have potential cost reductions of 43 cents per head for the small-size feedlot, 17 cents per head for

the medium-size feedlot, and 27 cents per head for the large-size feedlot. The overall reduction in costs amounts to about 15 percent. A large portion of the reduction is in labor costs and is due primarily to the compactness of the design which reduces travel for receiving, inspecting, feeding, cleaning, and loading out. Equipment costs also are reduced. Efficient and proper utilization of equipment for preparing feed and feeding cattle accounted for most of the reduction in equipment costs. Economics of scale showed the self-mixing, self-unloading truck method to be the lowest cost for feeding in feedlots ranging in capacity from 1,000 to 4,000 head; the mixing mill self-unloading truck (mill capacity 40,000 pounds per hour) method to be the lowest cost for feedlots ranging from 5,000 to 7,000 head; and mixing mill self-unloading truck (mill capacity 75,000 pounds per hour) method, the lowest cost for feedlots ranging from 8,000 to 10,000 head. This study has been completed and the project terminated.

## B. Automation of Sales Operations on Livestock Markets

At the Central Missouri Livestock Auction Market, Mexico, Mo., a test system for automating sales operations, consisting of a combination electronic load-cell and lever-system scale for weighing livestock, a scoreboard for flashing gross weight and average weights to the audience, a scoreboard for flashing price to the audience, a recorder for transferring sales information from the auctioneer's box to the office, a computer for receiving sales information in the office and preparing the seller's check, and electrically-operated pen gates were installed. The combination electronic load-cell and lever-system scale is a newlydeveloped scale for weighing livestock. The scale in the Central Missouri Livestock Auction was tested twice at about 4-month intervals by the Missouri Weight and Measure Division and the Packers and Stockyard Division. On each test, the scale tested out within specified tolerances and has been approved by these two Divisions. Preliminary tests show that when the scale is connected to a computer and a scoreboard, the gross weight and the average weight of a lot of animals are determined in about 5 seconds and flashed to the audience as compared with about 12 seconds when weights are determined by a conventional scale and average weights are calculated with hand-operated equipment. The reduction in the time for making weight determinations reduces the market's total selling time. The price scoreboard also worked satisfactorily. However, the computer failed to receive sale information from the auction box, make the necessary extensions, and prepare the seller's check fast enough to prevent delays in the sale. The computer functioned on about a 35-second cycle whereas a cycle of approximately 20 seconds is needed.

The problem is now being studied to determine what modifications or curtailments in the present plans for automating the sales operation are needed and what types of equipment should be used to accomplish this objective.

The electrically-operated pen gates failed to function with the degree of satisfaction desired. Although the gates opened and closed at the proper speed and stopped when coming in contact with animals without injuring them, when once stopped by animals during the cycle the gate could not again attain the desired speed for opening or closing promptly. The problem encountered will be further studied to determine if the defects in the present engineering design of the gates can be corrected within feasible cost limits.

# C. Determining Behavioral Patterns of Livestock

Because of inability to find a qualified contractor, no work was initiated.

# D. Developing a Physically Integrated Livestock Marketing and Slaughtering Facility

Work on this project to date has not progressed to the point that significant results can be summarized.

# E. Layout and Work Methods for Hotel Supply Houses

At Washington, D. C. a draft of manuscript entitled "Hotel and Restaurant Meat Purveyors Custom Service House - Improved Methods and Facilities" was completed. The studies show that the typical custom service hotel supply house handling 2,600,000 pounds annually could reduce its costs \$3.47 per 1,000 pounds by using the recommended work methods and plant layout. A reduction in cost would be incurred in all major operations. The largest reduction in cost is incurred in the loading-out delivery trucks. The cost for loading out is reduced from about \$1.77 per 1,000 pounds of meat and meat products with the typical method, to about 72 cents with the improved method. of the rack for loading out with the improved method reduces the amount of handling involved. A layout also is suggested for a house handling 2,600,000 pounds of meat and meat products annually, showing a possible arrangement of equipment and work areas, together with a suggestion for expanding the house to handle twice its planned volume. Field work on a similar study for portion control houses has been completed but the analysis of data has not progressed to a point where the findings can be summarized.

# F. Layouts and Work Methods for Cattle Slaughtering Plants

At College Station, Tex., working in cooperation with the State Station, a report entitled "Improving Methods and Facilities for Cattle Slaughtering Plants in the Southwest" was completed and published in 1961. Improved work methods and a plant layout can reduce costs for the typical plant slaughtering 100 cattle daily 50 cents a head or \$13,000 annually. This reduction is based on the typical dressed

weight of slaughter ratio of 50 percent of the carcasses weighing from 150 to 349 pounds, 40 percent from 350 to 599 pounds, and 10 percent from 600 to 900 pounds. Labor and equipment requirements vary with these weight groups. Following completion of this study, work was initiated at College Station, and a draft of a manuscript entitled "Cattle Killing Floor Systems and Layouts" was completed. The study compares the relative efficiency of the conventional bed-type, the gravity on-the-rail, and the powered on-the-rail systems. The costs for performing killing operations -- line dressing and supporting -- with the gravity on-the-rail and powered on-therail are about \$174.43 and \$177.19 per 100 head respectively, or so close that for practical purposes, their costs would be considered about the same. The cost per 100 head with the 3-bed system is \$203.56 or \$29.13 higher than the gravity on-the-rail system, and \$26.37 higher than with the powered on-the-rail system. The report also includes a suggested layout of a killing floor showing equipment arrangement, and work areas for each system.

A reduction in cost is incurred with the improved work methods and layout in all major operating cycles. The largest reduction in cost is incurred in the killing floor operating cycle. The cost for killing floor operations is reduced from \$156.69 per 100 cattle with typical methods and layout to \$118.87 with improved methods. A layout is suggested for a plant slaughtering 100 cattle daily. The layout shows the arrangement for the most direct flow of cattle carcasses, and meat products through the plant, and also a possible arrangement of equipment and work areas.

# G. Layout and Work Methods for Hog Slaughtering Plants

At Stillwater, Okla., in cooperation with the State Station, work was initiated in hog slaughtering plants similar to that completed in cattle plants. This study has not progressed to a point where the findings are significant.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

# Layouts and Work Methods for Commercial Cattle Feedlots

Webb, Tarvin F., May 1962. Improved Methods and Facilities for Commercial Cattle Feedlots. Marketing Research Report No. 517.

# Layouts and Work Methods for Cattle Slaughtering Plants

Hammons, Donald R., February 1961. Improving Methods and Facilities for Cattle Slaughtering Plants in the Southwest. Marketing Research Report No. 436.

### IV. ECONOMIC RESEARCH

# ECONOMICS OF MARKETING Marketing Economics Division, ERS

Within most agricultural processing industries rapid and Problem: drastic changes in their market organization and practices are occurring. These changes are affecting both farmers and consumers. Research is needed to keep abreast of such changes and to indicate their probable consequences. There have been substantial advances in recent years in increasing efficiency and reducing costs through adoption of new technology in producing, assembling, processing, and distributing farm products. However, for producers and marketing firms to remain competitive additional information is needed on margins, costs, economies of scale and efficiencies possible in the marketing of farm products. A significant aspect of the problem in marketing is that this type of information must be obtained from firms engaged in business -- in contrast with other types of research where the problem can be transferred to a laboratory, experimental plot, or other simulated situation. Consequently, it requires the cooperation of people engaged in making their living and assisting with marketing economic research on the side, where their own merchandise, facilities, and opportunity for profit and loss to themselves is involved. Another aspect of the problem is that only large firms can afford this type of research, consequently, public research has been requested for the many smaller firms. Furthermore, there is the need for comparison and analysis where even large firms do not have access to the plants and records of competitors.

Also, marketing research is increasingly directed toward evaluating present and prospective programs pertaining to agriculture such as the Food Stamp Program and Federal grading activities and to the changing structure of market industries. Changes in programs or market structure may influence the bargaining power of farmers. Marketing research also is being directed to the economics of transportation and storage activities of both private firms and government. Increasing attention is being given to the longer-term outlook for various products and markets as an aid in better assessing the prospects for increasing industrial employment under the Rural Area Development Program and in assessing prospective inter-regional shifts in the areas of production and marketing for specific products.

### USDA PROGRAM

The Department has a continuing program to determine the reason for the changes that are taking place in marketing so that ways can be found to increase the efficiency of the marketing system and make it more responsive to changing public needs. Because more than 50% of the consumer's dollar spent for meat products goes for marketing activities, this work encompasses a wide range of subject-matter.

It covers all economic aspects of marketing from the time the products leave the farm until they are purchased by ultimate consumers. Much marketing research is functional in nature and could apply to a number of commodities. To the extent the research effort devoted to livestock and livestock products can be distinguished, it is shown in terms of professional man-years in parentheses at the end of the following subareas:

## A. Market Potentials for New Products and Uses.

Continuing evaluations are needed of the commercial feasibility and market potentials of new or improved livestock products, appraisal of their impact on present markets, and of the economic and technical requirements of end uses. Such evaluation will provide a sound economic base for decisions on commercial development as well as information to guide further utilization research by physical scientists. (0.5)

## B. Merchandising and Promotion

This is research to evaluate promotional programs to determine:
(1) Responsiveness to advertising and promotional activities, and to identify characteristics of products that are responsive saleswise to promotion; (2) relative effectiveness of different promotional techniques or approaches when employed alone or in combination; (3) sales response-promotional investment relationship for selected products; and (4) organizational structure and procedures for optimum control, coordination and effective conduct of program. Another phase of the research program involves analysis of movement and availability data at retail and wholesale levels, and consumer purchase data by family characteristics including regions, rural and urban areas. This research delineates markets and provides producer groups information on movement and market profiles for specific products in planning and executing marketing programs.

Research is conducted to determine the influence on sales and consumer demand of merchandising practices and pricing policies characterising the marketing of specific commodities at the retail and wholesale levels of distribution. Evaluations are planned so that findings contribute to general principles and standards of performance relating to such factors as methods; type, location and size of displays; type, kind, color and size of package; variety and quality of products; and pricing techniques. Another basic area of research is designed to increase the efficiency of management through improvements in accounting procedures, inventory control, ordering, space allocation, and functional coordination between wholesalers and retailers, and thereby reduce costs associated with these items in the distribution of farm commodities. During the period covered by this report most of the work in this area was on lamb.

# C. Economics of Product Quality.

This program of basic and applied research on the economics of product quality includes study of the problems of seven different commodity groups. In addition, studies of cattle shrinkage are cooperative with the Colorado Agricultural Experiment Station. An early contribution made by government to improve the economic well-being of farmers was the establishment of standards for farm products. This assured the dependability of quality and strengthened farmers' competitive position. Knowledge of the impact of government grades on market practices and market structure provides a basis for answers to questions of public policy related to government grading programs. (2.5)

# D. Marketing Costs, Margins, and Efficiency.

This is a continuing long-term program. In nearly all studies, close cooperation is maintained with industry and trade groups and with individual private firms that provide essential data from their records and make their plant facilities available for observation in the conduct of various marketing tests. Much of the research is problemsolving in nature with a limited amount of research devoted to development of improved research techniques. Although much of the research is conducted by personnel in Washington, D. C., a considerable part of the work is done by USDA professional staff located in the States. (4.8)

# E. Market Structure, Practices, and Competition.

Market structure and practices research in the livestock industry is oriented toward the changing structure and marketing and pricing practices of wholesale meat distribution systems including meat packers, processors, wholesalers, and purveyors, and chain or independent retailers. It also includes an appraisal of the changes in market structure and pricing practices associated with the emergence of the commercial feedlot, as well as a study of the price formation relationships between country and terminal markets for hogs.

At Raleigh, North Carolina, Ames, Iowa, and Denver Colorado, cooperative research with the Southern, North-central, and Western regions is underway to project the locational changes of livestock and meat marketing institutions which are to be expected with the growh and locational shifts of population projected during the next 15 or 20 years. (5.7)

# F. Information, Outlook, and Rural Development.

This continuing program is designed to assist the Department in improving the usefulness of its output of marketing information through studies (1) to evaluate the uses made by both private and public users of information; (2) to determine the nature of the primary needs of these users for information; (3) to develop improved means of collecting data, making estimates, and reporting essential information; and (4) to evaluate impacts that information services have on decision making by farmers, marketing firms, and public and sem-public agencies. The need and adequacy of an experimental wholesale carlot meat market report is under analysis. The report is offered to a group of interior meatpacking points west of Chicago accounting for about 40 percent of the federally inspected meat in the United States. The professional man-years of effort specifically for livestock and livestock products is not available.

# RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

A brief but overall treatment of livestock research by States and industry is included in the "Introduction," beginning on page v. To the extent information was available the work by States and industry is reported under the following subareas with professional man-years shown in parentheses at the end of each section:

# A. Merchandising and Promotion.

Investigations are underway to find possible solutions of merchandising problems in the field of livestock and livestock products. Michigan is seeking the solution to technical problems in the merchandising of fresh and frozen meats. Missouri is studying measures to improve consumer acceptability of meats through higher quality resulting from breeding, feeding, processing, and cooking as well as to solve merchandising problems involved in kind of cuts being offered consumers, packaging, and methods of handling meats. Costs incurred in merchandising meat products with emphasis upon improved efficiency are being studied by West Virginia. The Kentucky station is investigating the relationship between processing costs and consumer acceptance of lightweight hogs. (4.6)

# B. Economics of Transportation and Storage.

Regional research by State Agricultural Experiment Stations is currently being conducted on the economics of transportation of livestock and livestock products in the Southern, Western and North Central regions. The research projects in this area represent well-integrated and coordinated State contributions to two regional research projects. In the South, with seven States participating, SM-23, An Analysis of Live-

stock and Meat Movement in the Southern Region, involves a study of meat and livestock movements which takes into consideration volume, direction and seasonal variation in movement, inefficiencies in movement, and the role of transportation costs and their implication upon the location of production and processing facilities. In the West, eight States are participating in regional project WM-37, of Transportation of Livestock and Meats in the Western Region. is concerned with an examination of the structure of rail and truck rates which prevail in the movement of livestock and meats, the equity of rates on inter- and intra-State movements, the costs and efficiency of shipping livestock and meats by truck and rail, and the effect of transportation costs on location of production areas and processing centers. Progress is being made toward combining forthcoming results of research in the South and West on the transportation of livestock and livestock products, with similar research to be conducted in the North Central region with perhaps some Northeastern State stations cooperating. In addition to the above, the Missouri station is making an analysis of rail and truck transportation costs for interregional shipments of livestock and meats, and Nevada is conducting research on controlled experiments in shrinkage resulting from transportation of cattle and sheep, and the cost of regain. (9.7)

# C. Economics of Product Quality.

In 1961 research on various aspects of the economics of quality and grade of livestock and livestock products was underway in the main livestock areas of the Nation. Several phases are covered by projects pertaining to the quality and grade of live animals and meats. In Illinois research was underway on the marketing of hogs on the basis of merit and value based on grading and pricing practices, and the retailing of pork on a graded basis. In New Jersey factors involved in preserving the quality of frozen and cured meats were being investigated. The effect of preslaughter handling of livestock upon carcass quality was being studied in Arkansas. In Oregon the work was directed toward developing quality prediction techniques for frozen beef based on carcass traits and consumer preference, and relating quality of beef to certain blood and liver constituents of the live animals. Work on lambs and wool also is included in the man-years of effort. (5.3)

Much of the industry work relating to the economics of product quality has been done by the meatpacking industry. Large national packers maintain taste panels to study consumer acceptance, evaluate brand and promotional policies, and market test new products. One packer has developed extensive studies of genetic and feeding factors affecting quality of meat and the relation of live animal characteristics to quality. One large packer has developed preslaughter tenderizing process by injection of meat tenderizer, for which effec-

tiveness is being tested. American Meat Institute Foundation is both studying and supporting university study of meat chemistry and value of meat in the diet and health. About 15 man-years are involved.

# D. Marketing Costs, Margins, and Efficiency.

A number of State experiment stations are engaged in research on costs, margins and efficiency in the marketing of livestock and livestock products. In the North Central States, research is centered around increased efficiency in meatpacking plants including optimum combination of products to process, quantitative techniques as a tool in decision-making in the marketing of livestock and livestock products; the potential expansion of livestock marketing and processing firms. The North Central States are also cooperating on a study, NCM-25, Adjustments in Livestock Marketing in the North Central States to Changing Patterns of Production and Consumption. In the Northeast, the stations are coordinating their research in a regional project, NEM-7, Factors Affecting the Efficiency of Livestock Marketing in the Northeast, studying factors associated with efficient buying, processing and distributing of livestock and meats. Southern stations are also coordinating their research in analyzing hog and pork movements, with a view to correcting inefficiencies in such movements, and increasing the efficiency of slaughter plant work methods and equipment, plant layout, design and location, SM-23, An Analysis of Livestock and Meat Movement in the Southern Region. In the West, the States are engaged in research to determine the economic factors affecting the economic location of markets and packing plants and the effect of different methods on costs and returns, WM-39, Alternative Marketing Methods for Cattle and Sheep in the West. (5.9)

# E. Market Structure, Practices, and Competition.

The State experiment stations, representing all regions, are conducting research on market structure and practices and their effect on the marketing of livestock and livestock products. Much of the effort takes the form on well-integrated and coordinated regional research. Midwest, ten States are participating in a regional project NCM-25, Adjustments in Livestock Marketing in the North Central States to Changing Patterns of Production. This research will indicate needed adjustments in production to prospective demand and will determine the effect of production, consumption and transportation costs upon the market structure of the industry. Five Midwestern States are completing regional project NCM-18, An Analysis of the Changing Pattern of Livestock Markets in the Corn Belt Region. The findings will provide information on marketing channels used by farmers and on practices followed and services performed by marketing firms. Various Northcentral stations are conducting research individually on the spatial and functional aspects of procurement, processing and merchandising facilities for livestock and meats,

with special attention to problems of location, structural changes, and economic factors influencing future marketing and processing. States in the Northeast are conducting regional research under NEM-7, Factors Affecting the Efficiency of Livestock Marketing, to determine the impact of specification production and buying upon livestock market organization and services. States in the South are cooperating in SM-23, An Analysis of Livestock and Meat Movement in the Southern Region, to determine the volume, direction and seasonal variations in movements and to determine their effect upon efficiencies of the livestock industry. Independently other Southern State research has to do with the effect of vertical integration upon livestock production and marketing; the impact of new and potential developments on market practices and the quality and supply of meats; and the effect of present organizational structure upon the marketing system. In the West, States are cooperating and participating on a coordinated basis in regional project WM-39, An Economic Analysis of Alternative Marketing Methods of Cattle and Sheep in the West. The analysis will show the nature and extent of direct marketing and costs and returns from different methods of marketing. Other research has to do with the impact of specification meat buying by large retailers on producer returns. (20.9)

# F. Information, Outlook, and Rural Development.

The adequacy of marketing information on livestock and livestock products is of continual concern to the experiment stations. Kentucky is making an economic analysis of livestock marketing information available in the State to determine what information is available on live animals and dressed meats to farmers and the meat-packing industry, and what additional information is needed to improve the accuracy of pricing these products. The Montana station is also conducting research for the purpose of appraising livestock marketing information available and to formulate needs of livestock producers in the State which when fulfilled will be most useful in decision-making relative to production and marketing problems. (2.2)

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE RESEARCH

## A. Market Potentials for New Products and Uses.

1. Market Potentials for Hides and Skins. Hides and leathers have been experiencing increased competition in the leather-consuming industries. Synthetics have been substituted in a number of uses and a new threat exists in the shoe market in the form of a leather-like synthetic being tested by a chemical firm. Analyses are being made of information obtained from suppliers and manufacturers to determine potentials for retaining leather markets through improved physical characteristics and qualities to meet market needs. In addition plans are being developed to appraise alternative markets to leather for raw materials from hides and skins, primarily collagen or gelatin.

2. Market Potentials for Fats in Feeds. Research on potentials for fats in feeds has been completed. Within the span of a few years feed use has developed into the largest single new market for fats and oils. It is predicted that this market will continue to grow in the next 10 years. Increasing numbers of feed manufacturers are adding fats to feeds and feed ingredients. As manufacturers gain experience in adding fats they are using fats in a wider range of products as well as increasing the level of fat added. Many advantages of adding fats to feeds has enabled this to develop into a major market outlet that has stabilized tallow and grease prices, particularly important in view of the displacement on other markets such as soap by synthetic materials. A wide range in kind and grade of fats and oils materials were found to be used with good results. Supply availability most frequently determines kind and grade of fat used.

# B. Economics of Product Quality.

- 1. Improved Live Hog and Carcass Grading and Pricing. The relationships of live and carcass weights, measurements, and evaluations to yields and quality characteristics of wholesale cuts and boned and defatted cuts were studied in order to establish a basis for (1) determining appropriate paying price differentials between grades, and (2) evaluating and improving the grade standards. In general, the current standards for barrow and gilt carcasses are quite good. The standards rely heavily on backfat thickness and weight or length. These factors are good indicators of the percent of lean cuts and percent of lean cuts, for a given weight or length, is very closely associated with value. Value differences between grades were computed under different price and grade consist situations so that paying price differentials actually used can be evaluated. (ME 2-33)
- 2. Grade Composition of Market Hogs. A reliable national estimate of slaughter barrow and gilt quality was needed as a basis for analyzing various aspects of the hog quality problem.

One grader spent a year visiting plants throughout the United States, which had been selected by systematic sampling methods. The plants in the sample were each visited twice. Of the barrow and gilt carcasses, 33 percent graded U. S. No. 1; 39 percent graded U. S. No. 2; 26 percent graded U. S. No. 3; 2 percent graded Medium; and less than one-half of 1 percent graded Cull.

It appears that I grader can spend enough days in enough plants to give adequate national estimates for a year's average consist. It was not possible to determine whether any region was definitely above or below the national average. At least 2 graders would be needed for acceptable regional and seasonal estimates. Other results of the study include distribution of barrow and gilt carcass lengths and backfat thicknesses by grades, and the distributions for sows.

A grader spent 2 weeks grading hog carcasses in Canada. The preliminary estimate of 1962 Canadian hog grade consist, in terms of U. S. grades, is 56 percent U. S. No. 1; 27 percent U. S. No. 2; 5 percent U. S. No. 3; 10 percent Medium; 1 percent Cull; and 1 percent Other. (ME 2-40)

3. Effects of Shrinkage on Pricing Cattle. Better knowledge of the shrinkage in cattle sold from feedlots, as compared with the arbitrary allowance or "pencil shrinkage" now used, will provide a basis for more efficient pricing. A study of the factors affecting shrinkage of fat cattle marketed from northern Colorado feedlots direct to packers and through the Denver stockyards will provide insights into this problem. No findings are available yet. (ME 2-30)

# C. Marketing Costs, Margins, and Efficiency.

In the decade 1949 to 1959, marketing margins for red meats increased sharply. Farm-retail price spreads for U. S. choice grade beef widened 57 percent, for pork 41 percent, and for lamb 45 percent. While margins for beef increased slightly again in 1960, margins on pork decreased about 9 percent.

Preliminary findings of an analysis of meatpacking costs in a sample of independent packinghouses indicate rising costs for slaughtering and cutting hogs and marketing fresh pork during a period when packer margins were narrowing. Additional detailed analyses are in progress on cost-efficiency ratios and labor requirements in slaughtering, cutting, boning, and shipping operations for pork and beef at representative plants.

In a comparison of prices of beef, milk, and eggs, and industrial workers' wages in the U.S. and the 12 countries that comprise the Organization for European Economic Cooperation, the labor time required to earn wages equal to the retail prices of these products was substantially lower in the U.S. than in all of the other countries with the exception of milk in 3 countries. The labor time required to earn enough to pay for a quart of milk in the U.S. and Sweden in 1955-56 averaged 7.1 minutes, 7.8 minutes for a laborer in Denmark, and 27.2 minutes for a worker in Italy. For eggs, the range in labor time was 19.5 minutes for a dozen eggs in the U.S. to 130.6 minutes in Italy. On beef, a U. S. worker had to work 20 minutes to earn enough to buy a pound of beef compared with 31 minutes in Denmark and 116 minutes in Italy. Thus, consumers in the U.S. fare much better than those in the OEEC countries when it comes to obtaining much for the money they spend on animal products. In general, farmers receive higher prices in the U. S. for beef, eggs, and milk than do farmers in most of the OEEC countries. However, retail prices and price spreads on these products generally were higher in actual amounts in the U.S. than in any of the other countries.

A study of pricing and operational efficiency of wholesale meat distribution in southern California suggests that price uncertainty and inadequate market information contribute to inefficient pricing and distribution of meat. Price variations within grades frequently exceed price differences among grades. Over half of California meatpackers are integrated with commercial feedlots, and packers sold about two-thirds of their output directly to retailers. Los Angeles wholesalers are specialized by volume and type of customer and specialization among independent packers is increasing. The competitive strength of large retailers relative to packers and wholesalers is increasing.

Important technological advances in methods of curing and handling hides have been made in the last 5 years. But many hide-marketing firms hesitate to adopt the new methods and invest in expensive new facilities without adequate information on the costs of curing hides by various methods. In response to this need a technical-economic analysis has been completed on 4 curing methods. Average total costs in 21 plants in 1962 were: \$1.87 per hundredweight (cured shipping weight) in plants packing salted unfleshed hides, \$1.59 in plants curing unfleshed hides by agitated brine methods, \$2.24 in plants curing fleshed hides by agitated brine and \$2.29 in plants curing fleshed hides by the pit cure method. Although pack-salt curing is the lowest cost method for plants curing fewer than 300 hides daily, the agitated brine is much less costly for plants curing 500 and more hides daily. Costs of fleshing hides drop sharply from 16 cents per hide at a daily volume of 400 hides to 12.5 cents at a volume of 1,000 hides a day. The research report on this study will contain many useful details on design of model hide-curing plants, facilities and labor requirements, and cost standards for efficiently operated plants.

# D. Market Structure, Practices, and Competition.

A number of econometric models have been constructed and tested in an attempt to describe the livestock-meat economy and to project current rates of economic change.

Trends in meat distribution by area from 1929 to 1958 show that changed relative importance of packer branch houses, wholesalers, and direct packer sales to retailers reflects closely the development of retail chains and the shift in population centers. Changes in patterns of consumption, distribution, production and processing of livestock and meat are being analyzed by regions for the U.S. The forces leading to these changes are being isolated and measured.

Substantial changes in the structure of the Southern Plains meat industry may be required in the next several years. Marked changes are occurring in population, incomes, retail grocery structure (growth of supermarkets and voluntary and cooperative retail groups), and in the technology of marketing, processing, and merchandising.

Hog and pork movements and hog price differentials in Six Southeast Coastal States have been analyzed to show that the least-cost optimal solutions, as a percent of actual hog movement costs, for one-week periods in November 1959 and February, May, and August 1960 were 78, 65, 64, and 67 percent, respectively.

Changes in method of sale in the West may affect (1) the structure of cattle prices, (2) costs and returns to feeders who sell to packers at the feedlot versus other methods of selling, and (3) the relation of the growth of large-scale commercial feedlots in the West to methods of sale and market structure for fat cattle.

Country market prices were higher for Good grade slaughter cattle than terminal market prices for the period of 1957-60. Terminal market prices for Choice grade slaughter cattle were higher than those at the country markets for the same period.

The consumers of the Southwest may prefer beef with less fat than the consumers in the Midwest and West. The country markets in the Southwest are stronger markets for Good grade slaughter cattle and the terminal markets in the Midwest and West are stronger Choice grade markets.

An econometric technique, consisting of a series of spatial-price equilibrium models with alternative assumptions on transportation costs and capital restrictions was used to evaluate the effect of changes in transportation costs on the location of slaughter. The model was tested with secondary data then used to generate evidence about adjustments that will be required to meet changes in the livestock and meat industries.

Changes from present transportation-rate structure based on value of product to one based on cost to the carrier of service furnished would decrease transport rates for meat relative to livestock. This would contribute to shifting of slaughter toward areas of surplus livestock supply and away from deficit areas and lead to shorter distances shipped for livestock and longer distances for meats.

Price relationships and price changes at terminal and interior country markets may disclose whether price leadership patterns exist among terminal and interior markets and provide a basis for more effective selection of market outlets by producers.

An analysis of day-to-day changes in hog prices indicates that changes are larger but less frequent at interior markets than at terminal markets. Market prices are more closely related within a market area in Iowa and Minnesota than between selected Western and Eastern Cornbelt terminal market areas. Correlation analyses of day-to-day changes in prices of hogs at the Chicago terminal market to other

terminal and interior markets indicate that there was a somewhat higher relationship between individual terminal markets and the Chicago terminal market than existed between individual interior markets and Chicago.

Numbers, sizes, and other characteristics of Southern California meat packers and wholesalers may be such as to explain some problems in supplying and pricing beef for that area. Southern California packers and wholesale meat distributors were asked about their supply sources, production, sales and inventories by price and grade, and retail food chains were asked about specifications, purchases, and price paid for meat by grade.

More than half of California packers were integrated with commercial feedlots. Packers sold two-thirds of the meat direct to retailers. Los Angeles wholesalers were specialized by volume and type customers. Packers' branch houses had decreased; number and specialization of independent packers and beef breakers increased. Changes in structure and meat wholesaling were influenced by development of chainstores and commercial feedlots. Competitive strength of large retailers has increased relative to packers and wholesalers because of their volume, specification buying, capital resources and alternative supply sources. Uneven purchases by retailers and uneven flow of feed cattle and carcass beef through marketing channels affected prices. Price uncertainty and inadequate market information contributed to inefficient pricing and price variation within grade often exceeded that between grades.

In the West, information on interstate and intrastate cattle movements has been assembled from brand and health inspection records. Movement patterns for feeder and fat cattle are being identified and shifts in the patterns are being analyzed. Attention is being given to the development of a framework for determining the factors important in feedlot and slaughter plant locations.

The geographic distribution by substate areas within the Southern region of livestock marketings, slaughter and meat consumption has been analyzed to show market outlets and sources of supply.

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# IMPROVING MARKETING OPERATIONS THROUGH RESEARCH WITH FARMER COOPERATIVES Marketing Division, FCS

Problem. Farmers, in marketing their production, face a revolutionary change in terms of market organization and marketing practices. The ever increasing and important supermarkets require large quantities, good quality, and frequent delivery which the small farmer, working alone, or a cooperative, or local firm of limited size cannot supply. Cooperatives must find ways to consolidate volume, either through internal growth, merger, acquisition or federation to help them meet the needs of mass merchandising. Ways must also be found to reduce marketing costs by increasing efficiency through improved operations, better organizations, and more mechanization.

Farmer cooperatives are an important part of the distribution system and represent a major potential for meeting the farmers' marketing problems in the modern distribution system. They are organized and operated to increase farmers' net income. Through cooperatives, farmers seek to increase their bargaining power; obtain needed services at cost; improve the quality of farm products; and obtain a larger share of the consumers' dollar. Cooperatives face many problems in achieving these objectives. Research is needed which will assist marketing cooperatives, as well as other marketing agencies, solve their problems by making available essential factual information and developing practical and useful operating plans and procedures.

#### USDA PROGRAM

The Department conducts a continuing long-range program of basic and applied research and technical assistance on problems of marketing farm products cooperatively. Studies are made on the organization, operations and role of farmer cooperatives in marketing. While most of the research is done directly with cooperatives, the results are generally of benefit to other marketing firms. The number of Federal professional man-years involved in this work totals 24.8 of which 3.5 are devoted to cooperative marketing of livestock.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State experiment stations are devoting 6 man-years to research on cooperative marketing. The work includes 1.5 on livestock and livestock products in Illinois to study corporate and operating structure of cooperative livestock associations, in Vermont to determine the extent to which farmers feel a need for a cooperative livestock auction or other form of marketing cooperative, and in Montana to study the potential of cooperative livestock feeding and marketing associations.

It is estimated that annual expenditures for research by cooperatives is equivalent to 11 man-years. This includes work on poultry and egg marketing, livestock marketing, including analysis of facility needs. Also included is research by farm supply cooperatives such as possibilities for integrated pork and beef programs, cow pools, consumer acceptance of frozen apple juice and prebaked beans, and feasibility of cooperative marketing of sugar beets.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

Efficiency of pooled sales. Part of the findings of this study were made public in a general publication on livestock pooling. Manuscripts covering feeder cattle and feeder pig pooling operations are completed and ready to go to the printer. Preparation of manuscripts covering lamb and veal calf pooling was started.

A total of 534 market agencies located in 33 states were pooling one or more species of livestock in 1959. Nearly three-fourths of these agencies were operating in the seven state area of Georgia, Kentucky, Missouri, Ohio, Tennessee, Virginia and West Virginia. Some agencies had been pooling for over 30 years but many started in the 1950s.

Market agencies indicated there were both advantages and disadvantages of pooling to themselves, consignors and buyers. The advantages far outweighed the disadvantages. Pooling reduces the market's operating costs and helps increase volume. Consignors receive a higher average price for pooled livestock and become better educated on livestock grades and the type and weight of animals buyers prefer. Market agencies estimated that pooling increased prices for feeder pigs from 25 cents to \$7.50 a pig and for feeder cattle and calves from \$1 to \$5 a hundred-weight. Pooling allows buyers to buy livestock in large, uniform lots and saves them buying time and expense.

Livestock integration. Preliminary information was released on the extent to which producers have integrated the production and marketing of livestock and meat through their cooperatives. Information has been provided to producer groups and cooperatives wishing to start integrated livestock operations.

The success with which a number of cooperatives have integrated their operations indicates there may be some opportunities for other producers to maintain control over their livestock and meat products further toward the consumer.

Improved market procedures in the Northeast. FCS has cooperated with the States in the Northeast region to find ways of improving livestock marketing. The findings to date indicate that too many livestock growers fail to sell at markets where price registers might be established. Most of the livestock is purchased by dealers from farmers, then dealers sell to slaughterers.

A second phase in this project studied labor utilization in slaughter plants. The results of this study showed that most slaughterers could lower their labor input per head of livestock handled at their plants.

Price differentials for hogs. This study shows that marketing agencies do not use USDA grade and price differentials between the grades to any extent. There are a number of cooperatives that sell hogs on a graded basis but they use their own standards, usually modification of USDA grades. A few agencies secure price differentials of 25-50 cents between grades 1 and 2.

Coordination of marketing. Studies have been made to determine the feasibility of livestock marketing cooperatives consolidating and combining operations to become a more important factor in selling of farmers' livestock. Nine cooperatives in the Middle West have joined together to form two organizations. This has increased their volume, overall efficiency, and improved services to farmer members.

In the South, studies have been conducted to determine the possibility of success of a new cooperative livestock market. It was found that the potential of success was present. Also in the Southern area a study to improve operations of a cooperative was carried out. It has been found that improvements can be made in management and services rendered.

Improving operating methods. Studies have been completed involving the analysis of the organization, operations and services of livestock cooperatives in California, Wisconsin and the Inter-Mountain States. The findings of these studies have alerted management to those segments of the cooperative that require strengthening to provide better services and to meet changes in marketing and production. The results of these studies not only help the specific cooperative association involved but serve as a guide for other organizations interested either in establishing or improving cooperative livestoc marketing agencies. Publications covering this work are in process.

Economics of slaughtering and processing livestock. It has been found that cooperative slaughtering and processing plants have the potential of increasing returns to growers. Some areas of the country should consider such plants while in others it appears that they would be uneconomical.

#### PUBLICATIONS REPORTING RESULTS OF FCS AND COOPERATIVE RESEARCH

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# ECONOMIC AND STATISTICAL ANALYSIS Economics and Statistical Analysis Division, ERS

Problem: Adequate and accurate information is needed on supplies, production and consumption of farm products, and the effects these and other factors have on the prices of agricultural commodities. Such information is needed in planning operations for the producers, processors and distributors and also benefits the consumer in selecting his purchases. Similarly, accurate quantitative knowledge of the interrelationships among prices, production and consumption of farm products are needed by Congress and the administrators of farm programs to effectively evaluate current and future price support and production control programs.

Due to the instability of the prices he receives, the farmer stands in special need of accurate appraisals of his economic prospects if he is to plan and carry out his production and marketing activities in an efficient and profitable way. The farmer needs to be provided with economic facts and interpretations comparable to those available to business and industry, through a continuous flow of current outlook intelligence and the development of longer range projections of the economic prospects for the principal agricultural commodities.

#### USDA PROGRAM

The Department has a continuing program of basic research concerning the factors affecting prices, supply, and consumption of principal agricultural commodities. This program has emphasized four broad research areas: (1) measurement of consumer response to price; (2) measurement of the effect of price and other factors on the production and supply of farm products; (3) measurement of the effect of supply and demand factors on farm prices and prices to consumers; and (4) development of statistical techniques for measuring economic relationship. The total Federal effort in this area is 8.0 professional manyears, of which 1.5 is concerned with supply, demand and price studies directly related to livestock.

This work is done in Washington, D. C. Research on livestock is part of a comprehensive analysis of the price-making forces in the feed-livestock economy. This study gives special attention to the quantitative measures that show what happens to the production of each commodity within the feed-livestock sector following changes in price of one or more of the commodities. The study includes analyses for the United States as a whole and for regions to measure differences in price response and to allow for the important farm and nonfarm alternatives available in each region. The emphasis during the past two years has been on factors affecting the price and supply of hogs. With completion of the hog study, research resources were shifted to a study

of the economic factors that affect the supply and price of beef cattle and the demand for feeder cattle and the interrelations among these factors. Results from the beef, hog and feed grain studies along with analyses for milk, eggs, and broilers will be incorporated into an overall analysis of the feed-livestock economy.

The total commodity situation and outlook program currently involves 24 professional man-years.

This includes the regular publication of 12 commodity outlook reports; the holding of the Annual Outlook Conference in Washington in mid-November; participation of outlook specialists at regional or State outlook meetings or at meetings of farm organizations and agricultural industry groups; preparation and publication of special articles bearing on both the short-run and long-run outlook for farm commodities; issuance of comprehensive statistical bulletins containing the principal economic series pertaining to the various commodities; long-range projections of the demand for the major agricultural commodities; and continuing analysis of the impact of various alternative farm program proposals as they affect output and prices of these commodities.

The work on livestock and meat involves 3.0 professional man-years in Washington and 2.0 professional man-years in Denver, Colorado. The outlook and situation program provides a continuing appraisal of the current and prospective economic situation of livestock and meats. appraisals are published 7 times a year in the Livestock and Meat Situation, quarterly in the Demand and Price Situation, and the National Food Situation. A comprehensive analysis of the livestock situation is presented at the Annual Outlook Conference. Outlook appraisals are frequently presented at regional or State outlook meetings, at meetings of farm organizations, and to various agricultural industry groups. Special analyses are prepared from time to time on the probable effect of proposed programs on the price, supply and consumption of livestock and livestock products. Basic statistical series are maintained, improved and published for general use in statistical and economic analysis. A Statistical Handbook, Livestock and Meat Statistics is published annually.

A Western Regional Field Office in Denver, Colorado, conducts a continuing appraisal of the conditions important to the range livestock industry of the West. The results of this activity are published monthly in the Western Livestock Round-Up, supplemented by special releases and special materials circulated to the Extension Marketing Specialists of the Western Region.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

The State experiment stations reported in 1961 a program involving 1.7 professional man-years. The work in Illinois was devoted to studying the influence of demand-supply relationship upon price decision making; in Iowa, the effect of product development on the demand for livestock; in Florida, on the relationship between prices paid for livestock in surplus producing areas and deficit market centers; and the Idaho and Washington stations were engaged in research to determine the factors influencing the demand and price of livestock and livestock products.

A few private colleges and organizations are engaged in price research, and may give attention to agricultural products from time to time. It is estimated that work on agricultural products may involve 5 to 10 professional man-years.

A substantial number of private organizations—including manufacturers of food and fiber products, private commodity analysts, banks, and investment houses—are engaged in commodity outlook work similar to that carried on by USDA. This work, however, frequently relates to shorter time periods than those covered by the Department's outlook appraisals; is predominately for private use; and not available to the public. Furthermore, much of the work of the private organizations is heavily dependent on the regular USDA outlook reports and the related statistical material. It is on the whole supplementary to that of the Department, rather than of a competitive or substitute nature. It has been estimated that this type of work in industry and other private organizations may total as much as 200 professional man-years.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

# A. Supply, Demand, and Price

Work on demand and prices for meat consisted of final preparation of a technical bulletin which summarizes some of the findings reported in a previous reporting period. This bulletin records the trends in production, consumption and price of the several meats and meat animals from 1921 to 1960. It seeks to ascertain values for economic factors affecting the demand and production of beef, pork and lamb are analyzed for three subperiods as well as the total period. Changes and similarities among these shorter periods are compared for a better understanding of short-term developments.

A study of the major economic factors that affect the supply and price of hogs has been completed and the results summarized in Technical Bulletin 1274, "Factors Affecting the Price and Supply of Hogs," which is now in press. Hogs are an important product for U. S. farmers,

contributing \$3.4 billion to farm income in 1961. And, hogs consume nearly half the annual corn crop. Fluctuations in hog production and prices have gone through two fairly regular recurring cycles since 1953. Some knowledge of these fluctuations would benefit both producer and consumer by eliminating overproduction and shortages. There are many factors that cause variation in the quantity of pork produced and the price received for it. The number of sows farrowing, the number of pigs raised per sow, the number of hogs slaughtered, their average weight, and storage operations of processors all affect the quantity of pork produced.

The hog study develops an explanation of the production and price cycles and measures the relative effect of various factors such as prices of feed, beef and poultry, and consumer income upon the important variables in the hog economy. The variables explained with regression analysis using quarterly data are: (1) the number of sows farrowing; (2) number of hogs slaughtered; (3) quantity of pork produced; (4) cold storage holdings of pork; (5) retail price of pork; and (6) farm price of hogs. Some of the findings from these analyses were that a 10-percent increase in the October-December corn price results in a 2-percent decrease in farrowings the following year. A similar increase in beef prices decreases farrowings by 1 percent. A 10-percent increase in the October-December hog price is associated, on the average, with a 4percent increase in farrowings the following year. The study also made a special analysis of seasonal variation in the hog industry. The analysis found that the amount of seasonal variation has gradually decreased during the last 10 years because of changes in feeding and production practices. In recent years, peak farrowings also occur earlier in the year than formerly.

The making of price, supply and consumption forecasts and the economic appraisal of alternative programs are two important related aspects of price analysis work. To implement this activity, a 20-equation model for the feed-livestock economy, recently published by G. E. Brandow of Pennsylvania State University in The Interrelations Among Demands for Farm Products and Implications for Control of Market Supply, has been revised to make it applicable to this work. One of the revisions included using a more recent base period--1960. The model was also revised to allow for changes in trends in consumption and price of those commodities which seem to be exhibiting different trends than they were when the model was originally formulated. The revised model is being used as one of the means in providing better estimates in projections work and economic appraisals of the Economic Research Service.

A study of the economic factors that influence the price and supply of beef cattle was recently begun. An analysis of changes in the regional patterns of production of different classes of cattle is underway. Preliminary results from this analysis indicate that formulation of regional supply functions for the various classes of beef cattle will be needed. This is based on the fact that certain factors affecting the supply of beef may be important to some regions, but not relevant to all regions. The influence of these special factors cannot be isolated in a statistical analysis for the United States as a whole.

# B. Commodity Situation and Outlook

The downward trend in beef prices that started in May 1959 came to an end in 1962. The increase in beef production this year was not sufficient to keep pace with the increase in demand, and fed steer prices reached the highest level in four years. Beef production is expected to increase in 1963 but not sufficiently to weaken prices. Relatively stable levels of pork production the past three years have been accompanied by gradually improving prices. Pork supplies in 1963 are likely to increase sufficiently to reduce prices slightly from the 1962 level.

A shift in the seasonal pattern of prices for several livestock commodities appears to have occurred in the mid-fifties. Current work includes an analysis of between 110 and 120 seasonal patterns of prices and production for various classes and grades of livestock and livestock products. Other work in progress is an analysis of the regional distribution of livestock production and the role of temperature on consumption of meat.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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- Harlow, Arthur A. 1960. The hog cycle and the cobweb theorem. Jour. Farm Econ., 42(4), pp. 842-853.
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- Van Meir, L. W. Livestock and Meat Situation. Published 7 times a year. ERS, USDA, Washington, D. C.
- Van Meir, L. W. June 1962. Meat imports rise in first quarter of 1962. Agricultural Situation.
- Van Meir, L. W. July 1962. The mid-year outlook for the livestock industry (mimeographed). ERS, Washington, D. C.
- Livestock and Meat Statistics. June 1962. Supplement to ERS Statistical Bulletin No. 230.

# CONSUMER PREFERENCE AND QUALITY DISCRIMINATION-HOUSEHOLD AND INDUSTRIAL Statistical Reporting Service

With the increasing complexity of marketing channels and methods, it has become almost impossible for the consumer to express to producers either his pleasure or displeasure with available merchandise. In order to market agricultural products more efficiently, we need to understand existing household, institutional, and industrial markets and the reasons behind cunsumers' decisions to purchase or not Information is needed on preferences, levels of information or misinformation, and satisfactions or dislikes of both present and potential consumers. We also need to know consumer attitudes toward the old and new product forms of agricultural commodities and their competitors, and probable trends in the consumption of farm products. We need to know the relationship between agricultural and nonagricultural products and the relationship of one agricultural commodity to another in consumers' patterns of use. Producer and industry groups and marketing agencies consider this information essential in planning programs to maintain and expand markets for agricultural commodities which, in turn, increase returns to growers.

#### USDA PROGRAM

The Special Surveys Branch of the Standards and Research Division conducts applied research on representative samples of industrial, institutional, or household consumers and potential consumers, in local, regional, or national marketing areas. Such research may be conducted to determine: attitudes, preferences, buying practices, and use habits with respect to various agricultural commodities and their specific attributes; the role of competitive products, and acceptance of new or improved products.

The Special Surveys Branch also conducts laboratory and field experiments in sensory discrimination of different qualities of a product. These studies ordinarily relate discrimination to preferences and attitudes as they influence purchases in order to assess the standards of quality, packaging, etc., which are needed to satisfy consumer demands.

In addition to surveys of consumer preferences and discrimination, the Special Surveys Branch also provides consultants and conducts special studies, upon request, for other agencies within the Department of Agriculture or within the Federal Government, when survey methods can be usefully applied to the evaluation of programs, services, or regulatory procedures of interest to the requesting agencies.

The work of the Branch is carried out in cooperation with other Federal governmental agencies, divisions within the Department of Agriculture, State Experiment Stations, Departments of Agriculture, and land grant colleges, agricultural producer, processor, and distributor groups. Closely supervised contracts with private research firms are used for nationwide surveys; studies in selected areas are usually conducted by the Washington staff, with the assistance of locally recruited personnel.

The Branch maintains all of its research scientists, who are trained in social psychology and other social sciences, in Washington, D. C., which is headquarters for all of the survey work whether it is conducted under contract or directly by the Branch.

The Federal scientific effort devoted to research in this area during the past year totaled 7.0 professional man-years under regular program funds; of this total, 6.8 professional man-years were devoted to consumer preference research and 0.2 professional man-years were devoted to quality discrimination research. An additional 2.2 professional man-years were devoted to research conducted under transfer of funds arrangements.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Agricultural Experiment Stations. The Stations do not report any of their work under this heading. However, they do have a considerable program in the area of consumer buying and use practices and motivation and decision making. This includes some research in preference and quality discrimination. There is a reference to parts of the stations' program in other division reports.

Industry and Other Organizations conduct research in this area, but the research done by individual firms and organizations is almost without exception for their exclusive internal use. There are very few instances in which the findings are made public or made available for government reference. In addition to the research actually initiated and paid for directly by industry, a substantial amount is undertaken in their behalf as part of the service provided by their advertising agencies.

Producer Groups. A number of food producer groups conduct consumer preference work with their own staff and, in addition, contract for research with private marketing firms. To a large extent, this research is limited to food classes such as poultry, dairy products, citrus and deciduous fruits (rather than being directed to individual branded products). This research ranges from a small to national coverage. It includes taste testing for quality differentiation, new product acceptance, and attitudes toward existing products. The producers of agricultural fibers have a small but active research program on quality evaluation and consumer preferences, both household and

industrial, for cotton and for wool. Estimated annual expenditures by these groups are equivalent to approximately 10 professional man-years.

Food Processors. In the livestock industry most of the larger packers have research programs for evaluating the effect of product change and acceptance of new meat products. A sizable number of other food processors have extremely large programs of consumer research. They are engaged in work on new food forms and convenience foods such as cake mixes, canned and frozen fruits and vegetables, deciduous fruits, citrus fruits, soups, dairy products, and alcoholic beverages. Manufacturers of cooking oils and shortenings support sizable consumer research programs with their own staff and also under contract. Manufacturers of dehydrated foods, such as potatoes, are constantly engaged in consumer research on their own and on competitors' products. Estimated annual expenditures are equivalent to approximately 400 man-years.

Processors of Nonfood Products. Large programs are supported by all of the major chemical manufacturers, directed to consumer preference and acceptance of synthetic fibers and blends. The three largest textile mills which represent a major proportion of the fabrics manufactured in the United States support research of this type. All of the large manufacturers of cigarettes do research to find out consumer taste preferences for their product; what blends, filters and packages are most likely to succeed in catching and holding a market. Estimated annual expenditures are equivalent to approximately 250 man-years.

Miscellaneous Groups. There is a smaller but constant amount of research undertaken by magazines and publishing houses for their principal advertisers. A number of the largest retail stores in our major cities study the consumers' reactions to their merchandise and service by conducting interview studies with customers and noncustomers. One of the largest food retailing chains has an active program in quality research which involves taste testing as well as consumer preference. Estimated annual expenditures for research related to agricultural commodities and nonagricultural products in competition with them are equivalent to approximately 20 professional man-years.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

Family food habits. A study of the effect of differences in national background, education, and occupational status of families on their food habits, done in cooperation with the Pennsylvania State University, was completed and a report has been issued by the University. This study was undertaken to ascertain the effect of ethnic and social characteristics of the family on the relative use of meats, dairy products, and certain fruits and vegetables. National background was found to determine to some extent the kinds and amounts of meats used,

although this influence appeared to decline among younger families. Families of American background were more likely to include fruits and fruit juices in their menus than were families of foreign background. Increased education and occupational status resulted in the increased use of fruits, fruit juices, nonleafy green vegetables, and cheese and ice cream, but not in the increased use of whole milk or meats and poultry. (MD 1-23)

Food stamp plan. The Special Surveys Branch participated in an evaluation of the pilot food stamp program at the request of the Food Distribution Division, AMS, which financed the research. Surveys were conducted in two of the eight pilot areas to determine attitudes about, and reactions to, the food stamp program. Groups interviewed were a cross section of low-income families who were, and also those who were not, participating in the program, moderate and higher-income families in the community, food retailers, and welfare workers. Results indicate general satisfaction with the program on the part of all groups. Retail grocers reported their sales increased. Participating families reported an increase in the amount and quality of the food that they consumed, especially in such products as meat, poultry, milk, eggs, and fresh fruits and vegetables. Welfare workers in the study areas were, in the main, satisfied that the program was doing a better job than existing or prior programs in improving the diet of low-income and needy families. Finally, the results indicated that families of moderate and higher incomes, although not themselves eligible for the program, had received the program with the recognition that low-income families should be enabled to obtain more and better food, and generally approved of the food coupon approach.

Preliminary results of these attitude surveys were included in a report of the food stamp plan which was issued by Agricultural Marketing Service early in 1962. A final report to Agricultural Marketing Service is in preparation. (MD 1-48).

PUBLICATION REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Food Distribution Division, AMS, USDA. 1962. The food stamp program: An initial evaluation of the program. Bulletin AMS-472 (MD 1-48).

#### V. NUTRITION AND CONSUMER-USE RESEARCH

Consumer and Food Economics Research Division, ARS
Human Mutrition Research Division. ARS

Problem. The assortment and characteristics of foods available to consumers are constantly changing with the adoption of new production, processing, and marketing practices. Constantly changing also, as nutrition science advances, is our understanding of the nutritional needs of man and the manner in which these needs can best be met by food. To help carry out the Department's responsibility to advise on the quantity and variety of foods that will assure maximum benefit and satisfaction to consumers, continuous research is essential on the nutritional requirements of persons of all age groups, and on the nutrient and other inherent values of foods and how to conserve or enhance these values in household preparation and processing. Periodic examinations of the kinds and amounts of foods consumed by different population groups and individuals also are essential for evaluation of the nutritional adequacy of diets and to give the guidance needed for effective nutrition education. Such information provides assistance needed in market analyses for different commodities and in the development and evaluation of agricultural policies relating to food production, distribution, and use.

#### USDA PROGRAM

The Department has a continuing program of research concerned with (1) nutritive and other consumer values of raw and processed foods as measured by chemical or physical means and by biologic response; (2) effects of household practices upon the nutritive volume and inherent qualities of foods, and the development of principles and improved procedures for household food preparation, care and preservation; (3) surveys of kinds, amounts, and costs of foods consumed by different population groups and the nutritional appraisal of diets and food supplies; and (4) development of guidance materials for nutrition education programs.

The research is carried out by two divisions of the Agricultural Research Service -- the Human Nutrition and the Consumer and Food Economics Research Divisions. Most of the work is done in Washington, D.C., and at Beltsville, Maryland; some is done under cooperative or contract arrangements with State Experiment Stations, universities, medical schools, and industry. The total Federal scientific effort devoted to research in these areas totals 61.1 man-years. It is estimated that approximately 13.6 man-years is concerned with studies related to livestock products.

Human metabolic studies and the related exploratory and confirmatory studies with experimental animals and micro-organisms concerned with defining human requirements for nutrients and foods are not reported on a commodity basis, though some of the work is applicable to this report. This basic nutrition research is described on a nutrient basis in the report for the Food and Nutrition Advisory Committee. The total Federal effort is 29.5 professional man-years.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Station research in 1961 included 22.4 professional man-years devoted to studies of the inherent properties of foods and of their household use; 17.6 to analyses of a variety of foods for vitamins, various lipid and protein components, and minerals; and 2.7 to studies of food consumption and dietary levels of households and of food management practices. Although the State work has not been reported on a commodity basis, some of the above research is applicable to this report.

Industry and other organizations such as universities and professional organizations are estimated to devote about 36 man-years to research on the preparation of materials for nutrition education, surveys of diets of individuals, and studies of functional properties and stability of food and of their specific nutrient contents. Limited work is done on the amount and structure of nutrients in foods and on compiling food composition data. Again, some of this work is applicable to this report.

#### REPORT OF PROGRESS OF USDA AND COOPERATIVE PROGRAMS

#### A. Nutritive Values of Foods

1. Tables of Food Composition. Data review has been completed for a revised edition of Agriculture Handbook No. 8, "Composition of Foods.. Raw, Processed, Prepared." This edition will have nearly 2,500 food items as compared with 75l items of the preceding edition, and upwards of 45,000 separate compositional values. For many foods, data will be provided for different forms--raw, cooked, canned, frozen, milled, dried, instant, dietetic, etc. The new publication will have, in addition to other constituents, data for protein, fat, carbohydrate, five vitamins (vitamin A, niacin, riboflavin, thiamine, ascorbic acid), six minerals (calcium, phosphorus, iron, sodium, potassium, magnesium). Explanatory notes for foods and nutrients will be added for users of the tables. Information on cholesterol and fatty acids will also be included.

A major expansion of the number of meats and meat products will be made in the revision of Handbook 8. Data will be included for about 175 beef items and beef products. For beef carcass, the composition

of six market grades will be shown in the new tables. Retail cuts, trimmed to the retail basis, will be reported for the two most important grades, choice and good, and for each grade as raw and as cooked meat. Data will be given for separable lean and separable fat tissue so that these can be combined in any desired proportions for individual needs. This will provide for variations from the average in retail trimming, and additional trimming in the home.

Data for pork will be shown in a similar way with similar subdivisions but the major classifications will be On the basis of fatness (fat, medium, and thin) rather than market grade. Fresh and cured pork items will amount to 130. Veal will also be classified by fatness rather than grade, over 30 items will be included.

Lamb will be reported under three grades, prime, choice, and good, with detailed information on the four major cuts trimmed to the retail basis. A total of 60 items will be included.

In addition to the major classes of meats, data on most of the edible organs (64 items) will be given, many both raw and cooked. A total of some 40 sausages, cold cuts, and luncheon meats will be included. Data for reindeer, venison, and a few other small game animals will also be reported (12 items). Miscellaneous mixed dishes including home-prepared and frozen dishes and dinners will be shown.

2. Vitamin Analyses. Values more representative of the vitamin B6 content of foods now may be obtained by use of a method recently developed at Beltsville, Maryland. Separation by column chromatography of the three forms of vitamin B6 naturally occurring in foods permit each form to be assayed individually. Values derived from these data for total vitamin B6 approximate closely values obtained from rat bioassay.

Research is in progress to combine a number of steps in the determination of various B-vitamins in order to facilitate their simulataneous analyses, permit complete characterization of the B-vitamins in foods and to determine their overall distribution in the food supply.

Livestock products. The vitamin B<sub>6</sub> value for dried lean beef muscle tissue as determined by rat bioassay was 13.2. Micrograms per gram as compared to 15.9 by the microbiological assay for pyridoxine, pyridoxal and pyridoxamine. The values for vitamin B<sub>6</sub> showed 55 percent pyridoxamine and 40 percent pyridoxal. On a fresh weight basis calves liver contained 5.4 micrograms; beef kidney, 4.5; frankfurters, 1.6; ham, 3.6. These studies are continuing.

Leg and loin cuts of lamb and of pork have been analyzed for thiamine, riboflavin, niacin, folic acid and pantothenic acid. These analyses were part of the studies on paired cuts, raw and cooked, for composi-

tion and distribution of nutrients in meats. The lamb data have been included in a manuscript for publication. A manuscript containing data on pork cuts is being prepared.

Pork muscle cuts contained less than 1 microgram of vitamin B<sub>12</sub> per 100 grams. Frankfurters, beef, veal and lamb muscle cuts contained 1 to 3 micrograms per 100 grams. Products having more than 10 micrograms vitamin B<sub>12</sub> per 100 grams of edible portion were liverwurst, 14; beef kidney, 38; lamb kidney, 63; lamb liver, 104; beef liver, 116. These data have been published.

3. Mineral Analyses. Leg and loin cuts of lamb and pork have been analyzed for content and distribution of mineral elements. These analyses are part of the studies on paired cuts, raw and cooked, for composition and distribution of nutrients in meats. Mineral content of separable lean and separable fat in raw cuts, calculated to milligrams of element per 100 grams of protein showed calcium, copper, iron and sodium were significantly higher in the separable fat on this basis than in the separable lean of both the lamb and pork cuts. A manuscript has been accepted for publication in the Journal of Agricultural and Food Chemistry.

The content of 10 mineral elements was determined in brain, heart, kidney, liver, pancreas (sweetbreads), and tongue of beef, and some in lamb, pork, chicken and veal. The data showed the iron content of the livers of swine and chicken was more than twice that of the livers from ruminants. By far the highest iron values were found in pork liver. Copper content of lamb liver was about three times that of beef or veal and 10 times that of pork or chicken liver. Sodium of pork pancreas was lowest among the organs studied. The data have been published and the studies terminated.

Foods were assayed for mineral element content using the emission of spectrograph for determinations of aluminum, boron, calcium, copper, iron, magnesium, manganese and phosphorus, and the flame photometer for the determinations of sodium and potassium.

Proximate Composition. Determination of the proximate composition of foods, i.e., moisture, fat, kjeldahl nitrogen and ash, were carried out in conjunction with studies for other nutrients in foods such as the vitamins, mineral elements, fatty acids and carbohydrates. Such an arrangement added to the information on composition of foods in the various commodity groups and also permitted the calculation of nutrients on a fat free-moisture free basis or on a nitrogen or protein basis where relationships among nutrients were concerned.

Proximate analyses of 24 pairs of hams are being made as part of a study on composition of cured and cooked hams. One ham of each pair was analyzed raw and the other baked for subsequent analysis. Both

baked and uncooked hams were separated into lean, subcutaneous fat, intermuscular fat, bone, waste and drippings (from baked hams). Data on the composition of paired raw and cured hams are being obtained under a contract at Urbana, Illinois.

Oven-baking of 3-ounce portions of pork sausage resulted in a higher cooked yield than did panbroiling; the amounts by weight of protein, fat and ash per patty were similar for both cooking methods but the moisture was higher in the oven-baked patties. The higher retention of moisture in oven-baked patties emphasized the need for basing serving portions on precooked weights rather than on cooked weights as now regulated for school lunch programs. The data have been published.

# B. Food Properties Related to Quality and Consumer Use.

1. Beef. The difference in cooking quality of beef cuts from different sources are being studied to obtain information from which to derive principles for modifying cooking procedures which will use to best advantage the qualities of beef available to the consumer. Palatability characteristics will be related to certain physical and chemical properties inherent in the beef cuts.

The comparative cost in terms of serving yield, preparation time and proximate composition of home prepared and purchased canned or frozen meat products were determined. Results are being evaluated and prepared for publication along with similar data on several types of food.

2. Pork. Studies were made at Beltsville, Maryland, and under contract at Ames, Iowa, and Urbana, Illinois, to determine the effect of carcass fatness upon composition, eating quality, and cooking performance of selected pork cuts. Thickness of carcass backfat had little influence on the degree of marbling, flavor, tenderness and juiciness of fresh pork cuts from the ham, loin and shoulder (Beltsville, Maryland; Ames, Iowa). There were indications that the tenderness and juiciness of roasts and chops might be related to the fat content of the longissimus dorsi muscle, but tenderness and juiciness of the cured hams did not show the same relationship to the fat content of the biceps femoris and semi-membranosus muscles. The amount of separable fat increased and of separable lean decreased with an increase in carcass backfat thickness, even though all cuts had been trimmed to packing house standards before yield determinations were made (Ames, Iowa). Muscles from carcasses with high marbling scores had more intramuscular fat and less moisture than those with low marbling scores (Beltsville, Maryland).

At Illinois, data were obtained on uncured hams from carcasses with low, medium and high thickness of cover fat and low and high marbling levels and on the paired hams after curing and smoking according to

common commercial practices. Factors measured include yield of lean meat, separable cover fat, separable intermuscular fat, skin and bone; proximate composition of lean meat, subcutaneous fat and intermuscular fat.

Yields of cooked meat from sausage containing different proportions of fat were determined and the number of lean-meat equivalent servings per pound were calculated for application to the National School Lunch Program. One pound of uncooked sausage having 33, 35, 37 and 50 percent fat content yielded 5.3, 4.9, 4.4 and 4.1 2-ounce servings, respectively, of cooked meat patties. The eating quality of sausage with lower initial fat content was as good or better than that of sausage with 50 percent initial fat content.

# C. Food Economics and Diet Appraisal.

1. Food Consumption and Dietary Levels. Information on the nutritive value of the food consumption of households based on the 1955 survey data has been summarized in Report No. 16 of the 1955 Household Food Consumption Survey series. Average family food supplies for a week in 1955 were sufficient to provide more than the National Research Council's recommended allowances for calories and eight nutrients for which values were calculated. However, many households (48 percent) had diets that did not fully meet the allowances in one or more nutrients. Other analyses of survey data show the relation of family size, the education of the homemaker, and of income to the food consumption of households. Because of interest in information on quantities of foods used by high consumers as well as average consumers estimates were made for some 60 food items of the ninth decile -- the figure dividing the highest 10 percent of the consumers from the lowest 90 percent. For the meat group, the amount consumed per person in "high consumption" households was nearly twice as much as in "average consumption" households.

A report of the food consumption and dietary levels of a group of older, low-income households in Rochester, New York, is in preparation.

Work is being undertaken on food consumption and nutritive content of diets of individuals. A systematic review and summarization of quantities of food consumed is being made through a cooperative agreement with the Minnesota Agricultural Experiment Station. A similar review of the nutritive content of the diets of individuals is being made by the Washington staff.

The nutrient content of the per capita food supply, calculated each year, using data on retail weight quantities of food as developed by the Economic Research Service, provides the only source information on year-to-year changes from 1909 to date.

2. Food Management Practices. Information on the kinds, amount, and nutritive value of foods used and discarded in households has been obtained in a series of small studies. Results will help to evaluate survey data on household food consumption.

A report on household practices in handling and storing of frozen food has been prepared, based on surveys in Baltimore, Maryland, and Indianapolis, Indiana. Households provided information on the length of time frozen food was held in home storage, and the temperature of the compartment in which frozen food was being held at the time of the interview.

3. Development of Food Budgets and Other Basic Data for Food and Nutrition Programs. An important aspect of nutrition research is the interpretation and application of research findings to practical problems of food selection in relation to health. An ongoing program of work includes assembling and interpreting available information on nutritional needs, food consumption, and nutritional value of foods for use by nutritionists, teachers, health workers, and other leaders concerned with nutrition education programs.

A technical report explaining the development of the food budgets, "Family Food Plans and Food Costs," has been completed and is in press. Another in the series of popular publications on food management has been prepared, "Food for the Young Couple." A publication, "Family Food Budgeting...for good meals and good nutrition," designed to help families of all sizes is also being prepared.

Regular pricing of family low-cost, moderate-cost, and liberal food plans is published in Family Economics Review on a quarterly basis for the U.S. average and on an annual basis for the regions and the low-cost food plan for the South. Each plan gives suggested quantities of food that will meet nutritional needs for each of 17 age and sex groups and for women during pregnancy and lactation so that household or population totals may be obtained.

Nutrition Committee News, a bimonthly periodical prepared for members State nutrition committees and other workers in nutrition education provides a channel for disseminating pertinent information and for reporting nutrition education activities. A Nutrition Education Conference sponsored jointly by USDA through its Nutrition Programs Service and by the Interagency Committee on Nutrition Education was held in Washington, D. C., January 29-31, 1962.

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#### VI. MARKETING SERVICE AND EDUCATION

MARKETING SERVICE WORK BY STATE DEPARTMENTS OF AGRICULTURE Matching Fund Programs, AMS

The objective of matching fund marketing service programs is to assist producers and marketing agencies in finding answers to their marketing problems through the application of research findings and improved marketing practices. Programs are being conducted in 43 States under the authorization of the Agricultural Marketing Act of 1946. The matching fund program in the States includes four broad fields, namely: (1) Improving or maintaining the quality of farm products; (2) expanding market outlets; (3) increasing marketing efficiency; and (4) collecting and disseminating new and improved marketing data.

The program is carried on by the States with financial support from the Agricultural Marketing Service on approved projects and is administered by a small staff office in the Agricultural Marketing Service. For more information see "Marketing Service Programs," a digest of activities by States, published annually for each year ending September 30, by the Agricultural Marketing Service.

# 1. Objectives of the Program for Livestock

This cooperative work is aimed at helping people solve problems that fall within the four broad fields of work cited above. For livestock, some of the problems to which this work is directed include determining (1) the best facility design for a particular marketing operation; (2) the most efficient equipment and plant layout; (3) the most effective quality control procedures; (4) improvements needed in grading, packaging, refrigerating, loading, transporting, and retail merchandising to reduce marketing costs and give the product greater consumer appeal; and (5) providing State and local data on supplies, prices, movement, and other pertinent information.

# 2. How the Program is Carried Out

Programs of this type are carried on under projects initiated by the States and submitted to the Agricultural Marketing Service for consideration and approval. If a project is approved, up to one-half of the cost is financed from the appropriation to AMS for "Payments to States and Possessions." Projects are reviewed annually to determine the results being achieved and the need and justification for continued support with matching funds.

The program places emphasis on personal contact with producers and marketing agencies in assisting them in finding solutions to their problems.

An important activity is helping to conduct special sales where buyers are able to purchase in quantity and small producers have an opportunity to market collectively through organized sales and pools. This procedure in turn provides an excellent opportunity for producers to become familiar with the application and usefulness of official grade standards. Meat merchandising schools are held; assistance is given in the development of facilities and efficient handling procedures; also assistance through this program makes it possible to have expanded data collection and reports.

# 3. Activities, Accomplishments, and New Developments

Twelve States conducted service programs on quality problems in the marketing of livestock. Eleven had programs on expanding market outlets. Six States conducted programs on increasing marketing efficiency. Sixteen States conducted programs which provided current basic data needed in making marketing decisions for livestock.

The following examples of State work will illustrate in more detail the content and procedure of the program in action:

Quarterly Estimates of Sow Farrowings - In each of the 10 States which issue the March 1 and September 1 estimates of sow farrowings to supplement the regular June 1 and December 1 reports, the activity is financed partly with matching funds. The States in this program are: Iowa, Kansas, Illinois, Indiana, Minnesota, Missouri, Nebraska, Ohio, South Dakota, and Wisconsin. The information provided in the reports on inventories, past and intended farrowings, has been of incalculable value to farmers in adjusting their hog marketings to market demands.

Specification Buying of Meat for Institutional Purposes in Kansas - In response to requests from State institutions and local packers, the Marketing Division of the Kansas State Board of Agriculture, in cooperation with the Livestock Division, AMS, initiated a pilot meat grading and certification project under the Matched Fund Marketing Service Program. This program greatly simplifies procurement problems of State institutions by enabling them to purchase by specification and at the same time permits State packers to compete for the institutional market. The specifications permit better portion control and lower preparation cost. As a result, State institutions are able to purchase meat products of uniformly higher quality at no increase in the per meal cost for the meat portion. In addition to the development of these specifications, the Marketing Division conducted meat cutting demonstrations for the benefit of procurement agencies.

Beef Carcass Evaluation Program Initiated in Michigan - To encourage production of beef tailored to market demands, the Michigan Department of Agriculture with Michigan State University and the Livestock Division, AMS, cooperating, inaugurated a service which provides beef cattle breeders and feeders with information on carcass characteristics of individual lots of cattle. Information obtained and furnished cooperating producers is the

marbling score, fat thickness, conformation grade, color and texture of lean, character and color of fat, and maturity. These data are obtained on a ribbed-out basis after all regular plant grading and rolling have been completed. Evaluations are made only on cattle for which accurate breeding and feeding data are available.

Feeder Pig Grading and Sales Program in Virginia - Under a matched fund marketing service project, the Virginia Department of Agriculture has been instrumental in creating a thriving feeder pig industry through a series of special sales. Feeder pigs marketed at these sales in accordance with grades developed by the Virginia department and the Agricultural Marketing Service bring nearly \$4 more per head than pigs sold through regular outlets. More than 50,000 pigs have been marketed at the 80 special sales held since the initiation of the project in late 1958. Cooperating in efforts to further improve standards for grades, the Virginia Agricultural Experiment Station is evaluating the relationship of various standards to rate of gain and feed conversion efficiency.

Meat-Type Hog Demonstration in Kentucky -During March 22-26, 1962, the Kentucky Department of Agriculture, in cooperation with the Extension Service, local vocational agricultural departments, the Tennessee Department of Agriculture, and the Federal-State Livestock Market News office at Louisville, staged the second annual hog carcass demonstration at Mayfield, Kentucky. The purpose of this demonstration was to assist producers in the area to evaluate the carcass quality and leanness of their breeding and commercial stock, with the ultimate goal of upgrading the quality of hogs marketed from the area. Seventy-five producers entered nearly 400 hogs in the event. These entries were judged on foot and these placings were correlated with carcass value after slaughter. The 63 entries finally certified for the carcass contest ranged in carcass value from \$19.58 to \$21.78 per 100 pounds, liveweight basis. The prevailing prices for No. 1, No. 2, and No. 3 hogs being purchased on a live basis were \$17.25, \$16.90, and \$16.50 per 100 pounds, respectively. The Reelfoot Packing Company of Union City, Tennessee, cooperated in making the cut-out determinations.

# 4. Future Needs and Flans

The requests for matching funds to work toward the solution of local problems are largely a reflection of local needs. The future scope and direction of the work, as in the past, will very likely be determined within the framework of the objectives aimed at solving problems within the four major fields of work set forth at the beginning of this statement.

# LIVESTOCK STATISTICS PROGRAM Agricultural Estimates Division, ERS

The purpose of the statistical and other reporting services for agricultural commodities of the Department of Agriculture is to provide accurate, timely, unbiased facts for use in appraising the situation and in making current and long range plans. Historically the statistical reporting service was started over 100 years ago to meet the need expressed by farmers to know as much about crop, livestock, and poultry supplies as the people to whom they sold. Today this type of information is widely used by farmers, processors, distributors, lending agencies, local, State, and Federal Governmental agencies and other agricultural interests. With the exception of the Bureau of the Census, in the Department of Commerce, which takes a census of agriculture every 5 years, no Governmental agency outside the Department of Agriculture provides any considerable body of official statistics pertaining to agriculture.

The reporting and statistical services include for a wide range of crop and livestock commodities, current national and State estimates of acreage, inventories, production, livestock slaughter, prices received and paid by producers, value of production and sales and related information. This service work is cooperative and depends to a large degree on the voluntary reporting of information by farmers and by the business men who deal with farmers. These public spirited individuals cooperate with the Department in pooling their information for the common good.

The organizational structure of the Statistical Reporting Service consists of the Agricultural Estimates Division, Field Operations Division, the Crop Reporting Board, and Standards and Research Division. Within AED are the following statistical branches: Livestock and Poultry, Dairy, Field Crops, Fruit and Vegetables and Agricultural Prices. FOD consists of 43 State statistical offices and the Survey Operations Group. The following branches are located in the SRD: Data Processing, Research and Development, Special Surveys and Statistical Clearance. For more information see "Major Statistical Series of the USDA, Volumes 1 and 8, Agricultural Handbook No. 118."

# 1. Objectives of the Program for Livestock

The aim of this reporting service on livestock is to furnish timely, comprehensive statistics by States and for the United States on inventory numbers, production, marketing, inshipments, slaughter, prices received, value, cold storage holdings of meat, and related items for use of producers, processors, distributors, consumers, and the general public. This involves collecting, compiling and analyzing data from many sources, such as individual producers, stockyards, packers, processors, handlers, cooperatives, warehouses, as well as from railroads and State and Federal regulatory agencies.

# 2. Procedure for Conducting the Program

Most of the basic information is collected, analyzed, and estimates are prepared by the State Statisticians. These estimates are reviewed by the Crop Reporting Board and the official estimates adopted. The Board issues releases showing the estimates by States and regions, and in turn the State Statistician issues reports which include supplemental information and details of local interest. In a substantial number of States, an agency, such as the State Department of Agriculture, or the College of Agriculture, cooperates with the U.S. Department in maintaining a joint statistical service for agriculture under the direction of the State Agricultural Statistician. In these States local area and county statistics and other special reports are prepared and published as part of the State program supplementing the basic Federal program, which is concerned primarily with State and national estimates.

# 3. Activities, Accomplishments, and New Developments

The established series of reports under this program were continued during the year and some of the reporting programs were expanded. Considerable attention has been given to modifying procedures in order to better meet the growing needs for statistical reports, and to keep up with changes that are under way. The demands by farmers, industry groups, government agencies, and others for more frequent, more comprehensive, more detailed and more precise reports have continued. Factors affecting the work are the dynamic changes that characterize the livestock industry, including increased specialization and commercialization and the declining importance of side line production.

Congress, in 1961, made additional funds available to expand the cattle on feed reporting program. In the cattle on feed report issued on October 1, 1961, two additional southern States were included in the quarterly program and one western State was added to the monthly program. Two eastern States were added to the January 1 report starting in 1962. Reports are now issued quarterly showing the number on feed by sex, length of time on feed, and weight classes as of the first of the quarterly period, the number marketed and the

number placed on feed during the preceding quarter, and marketing intentions for the following quarter for each of the 28 States. The January report shows the number on feed for 11 Southern and Eastern States. The monthly reports for three leading Western States include estimates of number on feed the first of the month and placements and marketings for the previous month.

The expanded cattle on feed program has met with favorable reaction from the livestock trade. The program has been successful in providing pertinent data to people interested in the livestock industry. Factual, unbiased estimates have provided valuable guides in determining the course for future operations. Few problems of consequence have been encountered, although additional work is continually needed to further improve the reliability of official estimates.

The project for improving methods of data collection was continued in 1962. Pilot studies were conducted in June and December in Southern, North Central, and Western States prior to 1961. Starting in 1961, the program was put on an operational basis in 11 Southern and 4 North Central States. Five additional North Central States were added to the operational program in 1962, and 4 additional States are expected to be operational in 1963. Pilot studies continued in 7 States in 1962. Further expansion of the operational program will be realized as additional funds become available. The results of the operational and pilot programs and related work are analyzed and modifications introduced in the operating program as their value is demonstrated.

At its February 1961 meeting, the Livestock Research and Marketing Advisory Committee recommended that increasing emphasis needed to be placed on developing a more comprehensive livestock statistical program to meet the increasing demands for statistical data and to keep the industry better informed on the changes that are under way. It was recommended that annual surveys be made to measure year-toyear changes in the number of producers of each species by size group strata; expansion of the quarterly pig crop report to include 18 States (8 more than at present) and to include estimates of pigs saved by quarters, as well as sows farrowed; more comprehensive marketing and livestock slaughter reports to include meat production estimates by States; and a survey to determine by States the number of each species of livestock by breeds. Congress appropriated funds during the last Congress that will permit Federal financing of the quarterly pig crop reports in 10 leading States, Ohio, Indiana, Illinois, Wisconsin, Minnesota, Iowa, Missouri, South Dakota, Nebraska, and Kansas. The expanded and improved program is designed to provide more information that will be useful to producers and to the livestock and meat industries. Some of the

major changes include: (1) Current inventories will be divided into "hogs for breeding" and "other hogs"; the latter category will be divided into five weight groups rather than into age groups. (2) The "pig crop" will be defined as the number of pigs farrowed during the previous quarter which are still on hand, plus those already sold. (3) Monthly estimates will be shown for the number of sows farrowed the previous quarter and the number to farrow during the following quarter.

# 4. Future Plans and Needs

Continuing effort will be made to keep up with the changes under way in the livestock industry. The expanded cattle and sheep on feed reports will be continued, and adjustments and modifications introduced in the basic reporting procedures to do this work more efficiently. Increasing requests from industry, governmental agencies and other agricultural interests have been made to develop further comprehensive statistical data to keep the industry better informed on increasing changes.

The Congress appropriated funds to be used as part of the long range program for strengthening and improving the overall Agricultural Estimates program. Improvement in livestock statistics can be expected as a part of this program, but except for the cattle on feed reports and pig crop reports, funds were not made available for new specific surveys in this area.

# 5. Publications

The livestock reports listed below are issued regularly by the Crop Reporting Board, U. S. Department of Agriculture, Washington, D. C. The 43 field offices of the Agricultural Estimates Division also issue reports for individual States. These State reports include local information and details supplementing the data included in the national release. In a substantial number of States, county statistics and other special reports are prepared in cooperation with a State agency. The following reports are issued regularly:

| Report   | Approximate release date |
|--|--------------------------|
| Cattle and Calves on Feed January 1, April 1, July 1 and October 1. Total number on feed,  | 16th of                  |
| by States; number on feed by classes, by weight groups, and by length of time on feed, and number placed and marketed during past quarter. | concerned                |
| Cattle and Calves on Feed Monthly report.  Number on feed, placements and marketings.  California, Arizona and Colorado.                   | 9th of<br>each month     |

| Livestock and Poultry Inventory, January 1. Number Value, and Classes by States.  | Feb. 13                                   |
|---|---|
| Calf Crop. Number of calves born during the preceding year, by States.  | Feb. 19                                   |
| Quarterly Pig Crop Report. Sows farrowing and inventory numbers, by weight groups, 10 States March 1, June 1, September 1, and December 1.  | Mar. 21<br>June 21<br>Sept. 21<br>Dec. 21 |
| Semiannual Pig Crop Report. Spring and fall pig crops, and farrowings indicated breeding intentions, by States.   | June 21<br>Dec. 21                        |
| Meat Animals - Farm Production, Disposition,<br>and Income. Data by States.   | Apr. 28                                   |
| Commercial Livestock Slaughter and Meat Production.  Number of head and live weight of cattle, calves, hogs, sheep and lambs slaughtered in commercial plants by States, Meat Production by species and Lard Production for the U.S.  Revisions by States and by months for previous year and total Livestock Slaughter, meat and | Issued last work- ing day of each month   |
| lard production, by quarters.   | April 28                                  |
| Western Range and Livestock Report. Condition of ranges, cattle and sheep, by States, Western States.   | 10th of<br>the month                      |
| Shipments of Stocker and Feeder Cattle and Sheep.  Number received in selected Corn Belt States, and monthly shipments from public stockyards.  | 25th of<br>each<br>month                  |
| Calf Crop. Number of calves born and expected to be born during the year, by States.  | July 22                                   |
| Cattle on Pastures in the Blue Stem (Flint Hills) and Osage Section of Kansas and Oklahoma  | June 12                                   |
| Prices Received by Farmers. Mid-Month prices received by farmers for livestock by species by States, indexes of prices received and paid by farmers and parity prices.  | 29th of<br>each<br>month                  |
| Wheat Pasture Report. Condition and availability of volunteer and seeded wheat acreage. South and Central Plains Area. Sept. 1, Oct. 1, Nov. 1, and Dec. 1.   | Issued about 12th of the month            |

### STANDARDS - CRADES - MARKET NEWS Livestock Division, AMS

Federal grade standards provide a system of market identification of quality differences in products widely identified by buyers and sellers. As trade progressed beyond simple barter, commonly used terms were developed for such factors as numbers, weights, measures, and prices. Grades represent a further step toward accurately describing product differences by identifying quality characteristics for which uniformly understood measures are not otherwise available. This identity is particularly important in the fast moving, modern business world, in which the buyer and seller often do not meet face to face. Standards for quality originally were developed primarily for the benefit of the producer in making production plans and marketing his products. They also have become increasingly useful to distributors in wholesale and retail trading and to consumers in buying.

Federal meat grading provides official interpretation and application of the U. S. standards for grades of meat. Meat grading began in 1927, largely through the efforts of the "Better Beef Association," an organization of cattle breeders and feeders. A major consideration in beginning the grading service was the feeling that labeling the quality of beef as an aid to consumers would provide a means for reflecting consumer preferences to producers. Market identification to aid in this expression of preferences remains an important reason for the existence of meat grading today.

Federal market reports for livestock and meats provide current market information on movement, supplies, demand, prices, and price trends. This service was begun in 1917. The need for a uniform and consistent system of reports by an impartial agent developed with improved transportation, refrigeration, storage, and communication facilities, which made previously local markets of importance nationally.

These marketing service activities for livestock and meats are conducted by the Livestock Division of the Agricultural Marketing Service in the Standardization, Meat Grading, and Market News Branches.

# 1. Objectives of the Program for Livestock and Meats.

The objective of standardization activities is the development of grade standards which are practical, meaningful aids in marketing. The application of more objective criteria which result in more precise market identification and which facilitate uniform interpretation and application of the standards is a continuing goal. The objective of the Federal meat grading program is to maintain uniform, consistent and impartial application of standards and thereby provide an accurate measure of quality differences in meat to aid orderly and efficient marketing. The objective of market reporting is to collect and disseminate timely and unbiased information on market conditions to aid producers and others in making informed decisions on marketing.

# 2. How the Program is Carried Out.

In the development of grade standards, close contacts are maintained with all segments of the industry in a continuing program of testing and development work designed to provide new or improved standards to meet changing needs. Research results are analyzed and adapted to practical use in standards, considering industry practices and changing production, marketing, and economic conditions. Proposed standards or revisions of standards are published in the Federal Register and comments received from the public are given careful consideration in deciding whether to make the proposals official. Work on standards for grades of livestock and meat is conducted at Washington, D.C.

Meat grading is a permissive service provided to qualified packers and other meat handlers on request. Fees are collected from these users to offset the costs of providing the service. The work is conducted at field locations at major livestock slaughtering and meat processing and distribution centers throughout the country. A supervisory staff at each of 16 stations is responsible for the technical and administrative aspects of the activity in the area. In addition, five National Technical Supervisors travel from one station to another to work with local graders and supervisors to assure that grading is done uniformly in all parts of the country at all times. Administrative and technical direction and coordination of the entire program is provided from the headquarters office in Washington, D. C.

Livestock market information is collected at terminal markets, auctions, production areas, and feedlots. Reports are released several times a day from each principal market—during trading hours and at the close of the day. A weekly summary also is prepared. Wholesale meat reports are prepared and released daily at major meat marketing centers and a weekly review is issued. In addition, local distributive meat trade information is prepared and released at several points. The work is conducted at 47 field offices, with each office responsible for collecting and disseminating information for a particular

market or area. Three area supervisors maintain close supervision of the various offices to effect uniformity in reporting practices and the application of official grade standards. The headquarters office in Washington, D. C. provides program direction and coordination and administrative support for the activity.

# 3. Activities, Accomplishments, and New Developments.

Decided consumer resistance to fat in recent years has prompted increased concern about the problem of excess fat in beef. Studies initiated by the Standardization Branch more than ten years ago were designed to identify the factors responsible for differences in yields of lean cuts and the magnitude of such differences. These studies revealed that beef carcasses and cattle with similar quality of lean meat vary widely with respect to yield of lean cuts vs. fat and other waste. In fact, some Choice quality carcasses may vary as much as \$100 in total retail value due to cutting yield differences. On the basis of the results of these studies, a new approach to grading beef carcasses and cattle has been developed. This has been referred to as dual grading. The dual grading system provides for separate identification of differences in the quality of the lean and in the yields of trimmed retail cuts from a carcass. The quality grade part of the dual grading system is practically identical with this aspect of the present grades. However, the yield grade part of the dual grade is given little consideration in the present system. The yield grades are based on a greater amount of detailed research and study than any previous development in grade standards for livestock and meat.

Refinement, field testing, and demonstration of the dual grading system have represented a major activity in recent years. Demonstrations and discussions have been conducted at numerous State, regional, and national meetings of industry groups, including cattle producers and feeders, marketing agents, packers, retailers, and research and extension workers. Also, field tests of dual grading have been conducted in cooperation with three packing plants to appraise application of the system under operating conditions. In addition, several lots of experimental cattle and carcasses have been evaluated on a dual grading basis in cooperation with State Experiment Stations. evaluation, measurement and cutting tests recently conducted in cooperation with the University of Illinois will provide further information on the application of dual grading and on the factors responsible for differences in cutting yields among wholesale beef cuts. Dual grading principles also are being used in placing carcasses in an increasing number of carcass contests and two major beef breed associations and Production Registry International have adopted carcass evaluation programs embodying the principles of dual grading. The new concept involved in dual grading makes extensive demonstrational and similar educational efforts vitally important to industry understanding of the system.

In response to industry requests for such action, the Secretary announced on April 10, 1962, that dual grading service would be made available for industry use on a trial and optional basis for a one-year period beginning July 1, 1962. This trial period was provided to give ample opportunity for close study and evaluation of the system. Conventional grading service for beef also is available during the trial period.

Closely related to the development of grade standards is the function of providing technical assistance in the interpretation and application of the standards for Federal meat grading and market reporting personnel. Such activity represents a continuing effort of the Standardization Branch to increase the effectiveness of these marketing service programs. Similar assistance is provided regularly to those conducting State livestock and meat grading programs and to research and extension workers. Consumer demonstrations of the selection of beef by grade and by cut are conducted regularly throughout the country in personal appearances and on television and radio programs. Detailed purchase specifications for meat products are developed for use in USDA and other governmental procurement programs as well as by institutional meat users.

In the Federal meat grading program, approximately 8 billion pounds of meat are graded or examined and accepted for compliance with purchase specifications each year. About 50 percent of the commercial production of beef is graded or accepted, as is 41 percent of the lamb and mutton, and 17 percent of the veal and calf. As of July 1, 1962, 436 graders were performing this voluntary service on a fee basis in nearly 2100 establishments throughout the country. The cost of this service averaged about 1/16 cent per pound of meat graded or accepted.

Three or four group meetings of field supervisors are held each year where intensive correlation grading work is done to insure continued uniformity of interpretation and application of standards. Program administration and management principles also are discussed at these meetings. Increasing emphasis has been given to training in the field of management, with about 25 supervisors participating in such training each year. In addition, area grader meetings are held to discuss mutual problems in the grading program. These activities have resulted in increased efficiency and improved work performance.

During the year which ended June 30, 1962, it is estimated that more than 60,000 individual market reports were prepared. This information was carried over USDA leased wire circuits throughout the country and widely disseminated by commercial wire services, trade publications, newspapers, and radio and television broadcasts. About 1 1/2 million summaries of reports were published and mailed to approximately 21,000 producers and others of the industry. The reporting service was expanded to include additional livestock auction markets in Pennsylvania

and to increase coverage of auctions and direct trading in Washington and Oregon. An expansion of service in 1960 involved a Federal-State agreement to provide reports of sales at livestock auctions and direct buying points in Illinois.

In addition to regular supervisory visits to field stations by area supervisors, six group training meetings for reporters were held last year. Emphasis was placed on maintaining uniform reporting procedures at all times and on the accurate and consistent interpretation of Federal grade standards used in reporting. State reporters also attended such training sessions in California and Oregon. One special training meeting was held for meat reporters.

# 4. Future Plans and Needs.

Future improvements in grade standards for livestock and meat will result primarily from the development and use of more precise and objective measures of grade determining factors. In the immediate future, the major emphasis in Standardization activities will continue in further refining and demonstrating the dual grading system for beef carcasses and slaughter cattle. Work also will be continued to extend this principle to feeder cattle, wholesale cuts of beef, and veal and calf carcasses. New standards for grades of feeder swine and cattle also are planned. The demonstrational program on all livestock and meat grade standards for various industry groups also will be continued, with possible increased assistance on State livestock and meat grading programs.

Meat grading activities are expected to continue at about the present level. Emphasis will continue on maintaining a high level of proficiency in accurately and consistently applying the standards. Improved efficiency and maximum utilization of manpower will be encouraged through continued attention to sound management principles and practices.

Although livestock and meat marketing patterns are changing, reporting terminal markets remains an important concern of the reporting service. With rather complete coverage of terminal markets achieved, future expansion likely will be in production area reporting of auction and direct buying points under Federal-State cooperative agreements. More emphasis on reporting volume meat transactions also is indicated by the increased effect of carlot meat trading on the livestock market. Continued attention also will be given to the uniformity of reporting procedures and the efficiency of operations.

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